The prevalence of abnormal glucose tolerance up to 5 years after an index pregnancy complicated by gestational diabetes defined using International Association of Diabetes and Pregnancy Study Groups criteria

Single Volume

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Publications and presentation arising from this work

Publications

Type 2 diabetes after gestational diabetes- the influence of changing diagnostic criteria (Review)
E Noctor, F Dunne
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Short- and long-term effects of gestational diabetes mellitus on healthcare cost: a cross-sectional comparative study in the ATLANTIC DIP cohort.
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ATLANTIC-DIP: prevalence of metabolic syndrome and insulin resistance in women with previous gestational diabetes mellitus by International Association of Diabetes in Pregnancy Study Groups criteria.
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ATLANTIC-DIP- Prevalence of abnormal glucose tolerance up to 5 years post gestational diabetes mellitus with use of International Association of Diabetes and Pregnancy Study Groups criteria
E Noctor, C Crowe, LA Carmody, J. Saunders, B Kirwan, JJ Infanti, A O'Dea, P Gillespie, Liam G Glynn, Brian McGuire, Ciaran O'Neill, PM O'Shea, FP Dunne [for] the ATLANTIC DIP investigators

Presentations

ATLANTIC-DIP- Comparison of fasting plasma glucose and HbA1c for follow-up of women with previous gestational diabetes

E Noctor, C Crowe, LA Carmody, B Wickham, P O'Shea, F Dunne *Poster Presentation, Irish Endocrine Society 37th Annual Meeting Irish Journal of Medical Science September 2012 181 S9*

ATLANTIC-DIP-The prevalence of pre-diabetes/diabetes up to 5 years post partum in women with previous gestational diabetes along the Atlantic coast

E Noctor, C Crowe, LA Carmody, B Wickham, G Avalos, G Gaffney, P O'Shea, F Dunne

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ATLANTIC-DIP-The prevalence of abnormal glucose tolerance up to 5 years post-gestational diabetes in a West of Ireland population

E Noctor, C Crowe, LA Carmody, B Wickham, G Avalos, G Gaffney, P O'Shea, F Dunne

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The prevalence of metabolic syndrome up to 5 years post-partum in patients with a history of gestational diabetes mellitus

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ATLANTIC DIP: The prevalence of pre-diabetes/type 2 diabetes in an Irish population with gestational diabetes mellitus 1-5 years post index pregnancy

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Validation of a diabetes risk score in identifying patients at risk of progression to abnormal glucose tolerance post partum
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Summary of contents

Introduction

Gestational diabetes (GDM) is associated with an increased future risk of type 2 diabetes, but the risk among women diagnosed with the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria is unknown. Therefore, we wished to determine the prevalence of abnormal glucose tolerance, metabolic syndrome, and insulin resistance in women with previous IADPSG-defined GDM.

Methods

We invited women with previous IADPSG-defined GDM up to 5 years post-partum for retesting with OGTT and HbA1c. A control group known to have normal glucose tolerance (NGT) at the same time also attended. Results were analysed using descriptive statistics, logistic regression, linear regression, decision tree analysis, and multidimensional scaling methods.

Results

270 women with previous GDM and 388 women with NGT in pregnancy attended (mean of 2.6 and 3.3 years follow-up respectively). 25.9% percent of women with previous GDM and 3.6% of women with previous NGT had AGT. Fasting and 2-hour glucose on pregnancy OGTT, gestational week diagnosed, family history, and BMI, were associated with AGT at retesting. The predictive power of the models was suboptimal. 25.3% percent of women with previous GDM and 6.6% with previous NGT had metabolic syndrome, while the prevalence of insulin resistance (HOMA2-IR>1.8) was 33.6% in women with previous GDM vs. 9.1% in those with NGT. Combining HbA1c ≥ 39 mmol/mol with FPG of ≥ 5.6 mmol/L yielded a sensitivity of 90% and specificity of 84% for detection of AGT.

Conclusions

IADPSG criteria for GDM identify a cohort at significant risk of metabolic disturbance up to 5 years post-partum. Identification of women at highest risk using routine clinical variables is not a useful strategy. Follow-up should be as frequent as for those meeting older GDM criteria. Combining HbA1c and FPG to detect AGT may be useful for longer-term follow-up.

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List of Abbreviations

ADA	American Diabetes Association
AGT	Abnormal Glucose Tolerance
ATLANTIC-DIP	ATLANTIC-Diabetes in Pregnancy (study)
ATP-III	Adult Treatment Panel III
AuROC	Area under the receiver-operator curve
BMI	Body Mass Index
CART	Classification And Regression Tree analysis
CI	Confidence Interval
DBP	Diastolic Blood Pressure
DPP	Diabetes Prevention Program (United States)
EASD	European Association for the Study of Diabetes
FINDRISC	Finnish Diabetes Risk Score
FPG	Fasting Plasma Glucose
GDM	Gestational diabetes
GIP	Glucose-Dependent Insulinotropic Polypeptide
GLP-1	Glucagon-Like Peptide 1
HAPO	Hyperglycemia and Adverse Pregnancy Outcome (study)
HDL	High-Density Lipoprotein
HbA1c	Haemoglobin A1c
HOMA2-IR	Homeostatic Model Assessment of Insulin Resistance (computer model)
HPL	Human Placental Lactogen
HSE	Health Service Executive (Ireland)
IADPSG	International Association of Diabetes and Pregnancy Study Groups
ICU	Intensive Care Unit
IFCC	International Federation of Clinical Chemistry
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance

IQR	Interquartile range
LDL	Low-Density Lipoprotein
MDS	Multidimensional Scaling
NDDG	National Diabetes Data Group
NICE	National Institute for Health and Care Excellence (Britain)
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NGSP	National Glycohemoglobin Standardization Program
NGT	Normal Glucose Tolerance
NPV	Negative Predictive Value
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
pGH	Placental Growth Hormone
PPV	Positive Predictive Value
RR	Relative Risk
SBP	Systolic Blood Pressure
SD	Standard Deviation
SMBG	Self-Monitoring of Blood Glucose
TG	Triglycerides
TNF- α	Tumour Necrosis Factor-α
WHO	World Health Organisation

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Chapter 1. Introduction

1.1. Aims

Gestational diabetes mellitus (GDM) is one of the most common medical disorders affecting pregnancy. The association of GDM with progression to type 2 diabetes in later life has long been recognised, having first been described almost 100 years ago. The subsequent detailed characterisation of the condition, and the introduction of diagnostic criteria in the 1960s, described later in this chapter, firmly established this association.

Despite the long-recognised association, this remains an area of important and clinically relevant research. The establishment of new, and now widely adopted criteria for GDM have increased the prevalence of GDM (as high as one in four pregnancies in some centres) as compared with older criteria. Therefore, from both a public health and individual patient perspective, determining the future implications of adoption of the newer criteria is essential.

Our main areas of clinical concern are;

1. What is the prevalence of glucose abnormalities in the medium-term in women with previous IADPSG-defined GDM?
2. What other metabolic risk factors are present in women with previous IADPSG-defined GDM?
3. Can we reliably predict which women with previous IADPSG-defined GDM are at highest risk of future abnormal glucose tolerance?
4. What is the best method of following women with previous IADPSG-defined GDM?

We will address our approach to these questions over the coming chapters. Firstly, however, we will describe the pathophysiology of GDM, and review the literature on progression from GDM to type 2 diabetes.

1.2 Pathophysiology of gestational diabetes (GDM)

1.2.1 Glucose homeostasis outside of pregnancy

In the non-pregnant state, serum glucose is maintained within narrow limits in the non-diabetic individual under normal conditions (range 3.9-7.4 mmol/L on interstitial monitoring)(1). Insulin and glucagon are the hormones primarily responsible for this. Glucose itself arises from just 3 sources;

1) Intestinal absorption of ingested glucose
2) Gluconeogenesis (synthesis of glucose from non-carbohydrate precursors)
3) Glycogenolysis (breakdown of glycogen stores to form glucose)

In the post-prandial state, glucose is abundant. Insulin secretion occurs in two phases-the first occurring 3-5 minutes after glucose ingestion(2) , augmented by the incretin peptides glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic polypeptide (GIP)(3). This first-phase insulin secretion acts predominantly on the liver, reducing hepatic glucose production both via the suppression of gluconeogenesis (indirectly via a reduction in glucagon secretion, decreased flow of precursors) and glycogenolysis (inhibiting glycogen phosphorylase). Second-phase insulin secretion occurs if serum glucose levels remain elevated, and acts predominantly on peripheral tissues, allowing uptake of glucose into muscle tissue, and to a lesser extent, adipose tissue, via the GLUT-4 transporter. From here, insulin promotes intracellular glycolysis and glycogen synthesis although this is predominantly carried out via inhibition of glycogen phosphorylase). The net effect of these measures is to lower the serum glucose level in the post-prandial state, thus returning it to the normal range. In the post-prandial setting, insulin also has significant effects on fat metabolism. Insulin activates lipoprotein lipase in adipose tissue, leading to hydrolysis of triglycerides in circulating chylomicrons; stimulates the

esterification of free fatty acids in adipocytes (via increased glucose transport, providing more substrate); and inhibits lipolysis in the adipocyte via the inhibition of hormone sensitive lipase. Insulin secretion also directly inhibits glucagon secretion, which is also indirectly affected by hyperglycaemia (via the action of somatostatin). The kidney also plays a key role in glucose homeostasis, reabsorbing all of the filtered glucose in the proximal tubule, 90% of which occurs via the SGLT2 (sodium glucose cotransporter) transporter. The remaining 10% occurs via the SGLT1 transporter(4).

During the fasting state, cells with glucose-dependent tissues (e.g. central nervous system) still require a constant supply of glucose. Thus, it is necessary for the body to maintain serum glucose levels despite the fasting state. In the fasting state, insulin levels are low. This has three main effects; firstly, insulin-dependent peripheral (muscle tissue and adipose tissue) uptake of glucose decreases (usually mediated via GLUT-4); also, the insulin–mediated suppression of both gluconeogenesis and glycogenolysis is reduced, thereby increasing hepatic glucose output. These measures have the net effect of maintaining a steady serum glucose level despite absence of intestinal absorption of glucose. In the fasting state, also, the reduction in insulin secretion permits ketogenesis due to increased fatty acid delivery to the liver resulting from increased lipolysis. The removal of the suppressive effect of insulin on the alpha cells also allows increased glucagon secretion, which contributes to ketosis(5).

1.2.2 Glucose homeostasis during pregnancy

Pregnancy imposes significant changes on normal glucose metabolism (Fig 1.1a). The foetus utilises glucose as a primary energy source, but has little capacity for gluconeogenesis, and is therefore dependent on maternal plasma glucose to meet its needs. Glucose transfer from mother to foetus occurs via the GLUT-1 transporter, and is determined by maternal plasma glucose concentration and placental blood flow. This extra requirement for glucose imposes several changes on glucose homeostasis. Fasting plasma

glucose levels are lower than in the non-pregnant state, (despite increased endogenous glucose production of up to 30%(6) – a relationship that is incompletely understood), while fasting insulin levels are also reduced (by approximately 50% -a possible mechanism for the increased hepatic glucose production, although this persists throughout pregnancy, despite rising insulin levels)(7). This combination leads to the state of accelerated ketosis seen during periods of fasting in pregnancy.

As against this, serum levels of progesterone, prolactin, and human placental lactogen are all significantly elevated (8). These may induce hyperphagia, while human placental lactogen also induces beta cell hypertrophy and hyperplasia, and as a consequence, increases insulin secretion. The combination of increased insulin secretion and unchanged insulin sensitivity in early pregnancy leads to normal or decreased glucose levels(9). This milieu changes, however, as pregnancy advances, and insulin sensitivity decreases. The change in insulin sensitivity is quite marked, and is decreased by 56% by 36 weeks gestation. This change parallels both the increase in placental size, and in the secretion of human placental lactogen, which peaks at 30 weeks gestation(10). Human placental growth hormone(10), progesterone(11), and the increased visceral adiposity resulting from increased fat deposition in early pregnancy, have all been described as contributing factors also. The renal threshold for glucose is also reduced in pregnancy, which may result in glycosuria, despite normal glucose levels (12)

The net effect is that, although glucose levels in pregnancy tend to be 10-20% lower than the non-pregnant state, increasing insulin resistance in the second and third trimester, along with increased hepatic glucose production, combine to provide a mechanism by which gestational diabetes may occur. Leptin and tumour necrosis factor-α (TNF- α) may also play a role, as yet incompletely characterised, in decreased insulin sensitivity.

1.2.3 Glucose homeostasis in GDM pregnancy

Despite the above changes seen in the normal pregnancy, the majority of women do not develop glucose intolerance. So why do some women develop hyperglycaemia as a result of these physiological adaptations?

In the non-obese pregnant woman without diabetes, the insulin resistance described above is compensated for by an appropriate increase in insulin secretion (9) to maintain normal plasma glucose levels (mean fasting interstitial glucose 4.0 mmol/L , SD 0.7; mean peak postprandial glucose 5.9 mmol/L, SD 0.9), although this is higher in obese women without diabetes(13).

The relationship between insulin sensitivity and insulin secretion, is however, nonlinear (best described by a hyperbola)(14). When insulin secretion is insufficient, for whatever reason, to overcome the insulin resistance induced by pregnancy (in addition to any predisposing factors that may be present), hyperglycaemia ensues(15) (Fig 1.1b).

The underlying beta-cell problem is most commonly associated with chronic insulin resistance and obesity(16, 17), although autoimmune(18) and monogenic(19) types of diabetes may also present initially as GDM. Whatever the aetiology, this underlying beta-cell dysfunction explains why compensatory insulin secretion may be sufficient earlier in pregnancy, but becomes inadequate as insulin resistance progresses with progressing gestation. This occurs along a continuum with no threshold effect. Therefore, the question remains of where to draw the line in differentiating between normal and abnormal states of glucose tolerance remains. This has been the subject of intense debate since the recognition of GDM, and I will discuss this in some detail in the following pages.

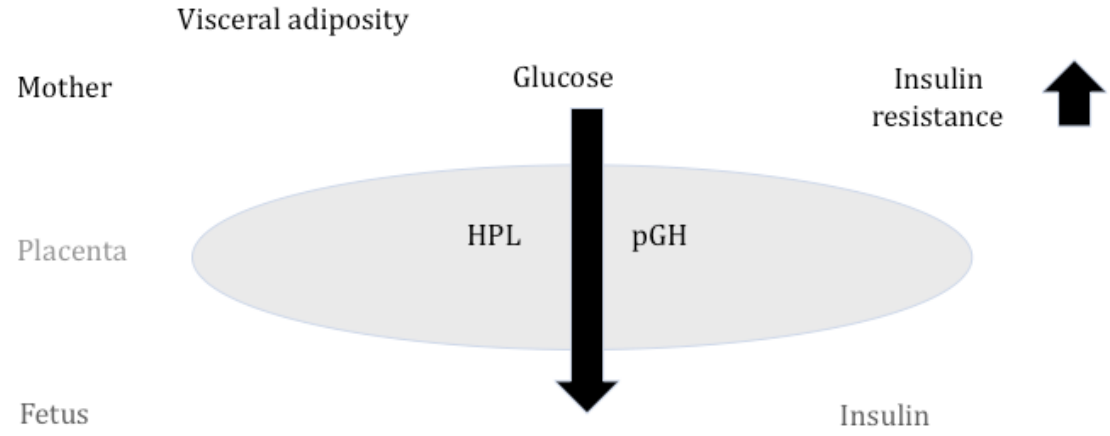

Fig 1.1a. Simplified schematic representation of glucose metabolism in normal pregnancy. Glucose passes freely through the placenta, while insulin does not. The foetus begins to produce its own insulin from the end of the first trimester. Human placental lactogen (HPL) and placental growth hormone(pGH) are produced by the placenta, and along with the visceral adiposity seen in pregnancy contribute to the increasing insulin resistance seen as pregnancy progresses.

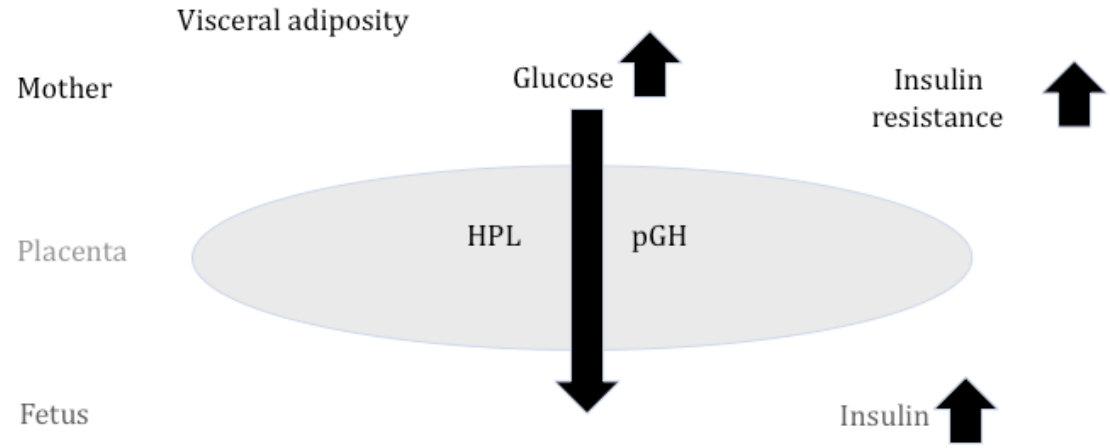

Fig 1.1b. Simplified schematic representation of glucose metabolism in pregnancy complicated by gestational diabetes (GDM). In women with GDM, insulin secretion increases, but cannot compensate for the increasing insulin resistance associated with advancing gestation. Maternal glucose levels rise, while foetal insulin levels increase to compensate for this (Pedersen hypothesis)

1.3 GDM diagnostic criteria

1.3.1 Establishing diagnostic criteria for GDM

Gestational diabetes (GDM) has long been recognised clinically. First described in 1824 in Germany by Heinrich Bennewitz, (20), Joslin also described in 1916 a case of glycosuria which presented in pregnancy, resolved with delivery, recurred in subsequent pregnancies, and progressed to permanent diabetes later in life(21). In the 1940s and 1950s, Hoet recognised the association of this type of diabetes with adverse perinatal outcome, and biochemically characterised the relationship between glucose tolerance during pregnancy, and in the post-partum period(22). However, despite the long-recognised association, no standardised criteria for diagnosis were devised until 1964.

This heterogeneity, both in methods used, and criteria applied, to diagnose GDM led to a reappraisal of the diagnostic process in 1964 by John B O'Sullivan, a Royal College of Surgeons of Ireland medical graduate working in Boston, and Claire Mahan, a statistician. Dr O'Sullivan published a series of 752 women (60% white, 40% black) attending Boston City Hospital, which was not a tertiary referral centre, in the mid-1950s for antenatal care. Of 986 women invited to participate, 752 (76.3%) attended. Analysis of 50g glucose challenge test results, which all 986 women underwent, showed no significant differences in plasma glucose levels, age or parity between those women who attended for oral glucose tolerance test (OGTT), and those who did not, excluding participant bias. Participants then underwent a 3-hour 100g oral glucose tolerance test, with plasma glucose values taken at zero, 1, 2 and 3 hours. Women with 2 out of 4 values that were greater than 2 standard deviations above the mean glucose levels (and rounded to the nearest 5mg/dl) determined in this paper were thereafter diagnosed with GDM. The requirement for two abnormal values was to avoid reliance on a single abnormal value for diabetes diagnosis.

These criteria have continued in clinical use over the following four decades, albeit in slightly modified forms.

The major feature of these criteria was that they defined a cohort of women with a greatly increased future risk of progression to type 2 diabetes, demonstrating a lifetime risk of up to 60%(23). The National Diabetes Data Group (NDDG) criteria, proposed in 1979(24) (Table 1.1), converted the O'Sullivan/Mahan criteria from whole blood to plasma values (see Fig. 1.2 for timeline). The Carpenter-Coustan criteria(25) also converted the O'Sullivan/Mahan criteria to plasma values, but in addition, took a change in enzymatic methods into account. These criteria were proposed in 1982 and soon entered widespread clinical use. Essentially, therefore, all 3 sets of criteria were intended to define a similar population.

Studies directly comparing the prevalence of GDM by either NDDG or Carpenter-Coustan criteria show, however, significant differences, with a greater than 50% relative increase in GDM prevalence if the Carpenter-Coustan criteria are used(26). In addition, in 2001, the American Diabetes Association (ADA), having previously endorsed the Carpenter-Coustan criteria, also allowed for the use of a 75g, 2-hr OGTT to make a diagnosis of GDM, using the same one- and two-hour cut-offs as the three-hour 100g OGTT.

Criteria	Glucose load	Fasting Glucose mmol/L (mg/dl)	1-hour glucose mmol/L (mg/dl)	2-hour glucose mmol/L (mg/dl)	3-hour glucose mmol/L (mg/dl)	Number of criteria required
O'Sullivan & Mahan	100g	5.0 (90)	9.2 (165)	8.1 (145)	6.9 (125)	≥2
NDDG	100g	5.8 (105)	10.6 (190)	9.2 (165)	8.1 (145)	≥2
WHO 1980	75g	8.0 (144)	N/A	8.0 (144)	N/A	≥1
Carpenter & Coustan	100g	5.3 (95)	10.0 (180)	8.6 (155)	7.8 (140)	≥2
ADA	75g or 100g	5.3 (95)	10.0 (180)	8.6 (155)	7.8 (140)	≥2
WHO 1985	75g	7.8 (140)	N/A	7.8 (140)	N/A	≥1
EASD	75g	6.0 (108)	N/A	9.0 (162)	N/A	≥1
WHO 1999	75g	7.0 (126)	N/A	7.8 (140)	N/A	≥1
IADPSG GDM	75g	5.1 (92)	10.0 (180)	8.5 (153)	N/A	≥1
IADPSG overt diabetes	*None/75g	7.0 (126)	N/A	11.1 (200)	N/A	≥1

*****_This diagnosis can also be made on a random glucose sample, a fasting glucose sample, or on an HbA1c value (if 6.5%- 48 mmol/mol- or over)_

Table 1. 1. Comparison of thresholds for criteria for gestational diabetes (GDM) diagnosis. NDDG- National Diabetes Data Group, WHO- World Health Organisation, EASD- European Association for the Study of Diabetes, ADA- American Diabetes Association, IADPSG- International Association of Diabetes in Pregnancy Study Groups

The post-load glucose levels are estimated as being 0.9 mmol/l lower at one hour, and 0.5 mmol/L lower at two hours with the lower glucose load(27), therefore these criteria will identify a slightly different group of women.

The World Health Organisation (WHO) also recommended alternative criteria for the diagnosis of gestational diabetes beginning in 1980 (the 1965 WHO report did not comment on this issue). These thresholds were the same as those for non-pregnant adults. Initially, the WHO recommended a fasting glucose threshold of 8mmol/L (see table 1.1). These recommendations were revised again in 1985(28) (fasting glucose threshold lowered to 7.8 mmol/L, recommendation to treat impaired glucose tolerance – a 2-hour post 75 g glucose value of 7.8 mmol/L or greater - added) and 1999(29) (fasting glucose threshold reduced to 7.0 mmol/L) (see table 1.1). The European Association for the Study of Diabetes (EASD) also proposed new GDM criteria in 1996(30), using a fasting value of 6.0 mmol/L and a two-hour post 75g glucose load value of 9.0 mmol/L, based on the distribution of glucose values on 75g OGTTs on over 1000 European women.

In addition to these major criteria, multiple different diagnostic criteria are in use worldwide, some related to older criteria, some derived on the basis of local data. Therefore, the situation still exists where different centres in the same country, or even the same region may employ different criteria for GDM diagnosis.

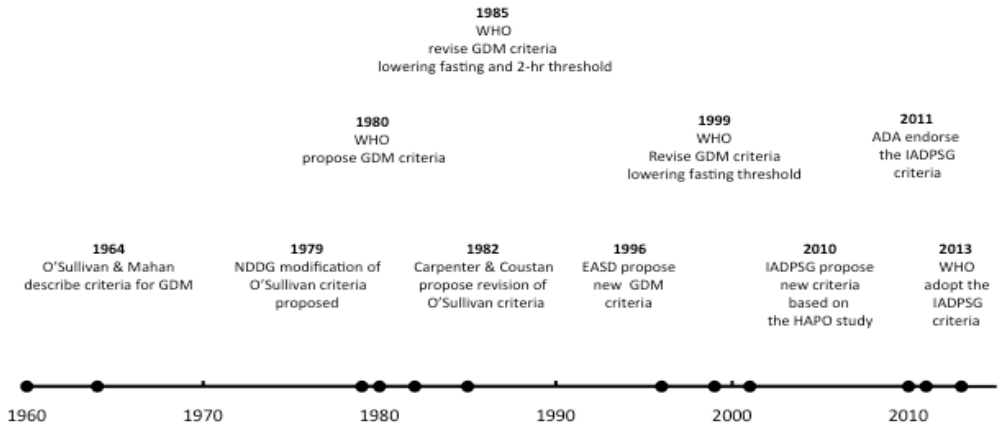

Fig 1.2. Timeline of evolution of criteria used to diagnose gestational diabetes (GDM) from 1964-present.
NDDG- National Diabetes Data Group, WHO- World Health Organisation, EASD- European Association for the Study of Diabetes, ADA- American Diabetes Association, IADPSG- International Association of Diabetes and Pregnancy Study Groups

1.3.2 GDM criteria to predict adverse perinatal outcome

However, none of the available criteria had been designed to predict adverse pregnancy outcome. To look specifically at this issue, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) convened a consensus conference in 2008 to review the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study findings (published and unpublished), along with other relevant studies.

Hyperglycaemia and Adverse Pregnancy Outcomes Study (HAPO)

The HAPO study was a multicentre observational study, carried out across nine countries, and was initiated in 2002 to answer the question of whether

glucose levels less than those diagnostic of diabetes were harmful to mother and foetus. 25,505 women were tested with a 75g OGTT. Pre-specified primary outcomes were primary Caesarean section, birth weight greater than the 90th centile, cord C-peptide greater than the 90th centile, and clinical neonatal hypoglycaemia. Prespecified secondary outcomes were preterm delivery (before 37 weeks), shoulder dystocia, neonatal intensive care unit admission, hyperbilirubinaemia, and pre-eclampsia. 23,316 participants met the inclusion criteria of fasting glucose 2.5-5.8 mmol/L, 1 hour glucose > 2.5 mmol/L, and 2 hour glucose 2.5-11.1 mmol/L. The final results were published in 2008 (Table 1.2), and showed that glucose levels at all time points on the 2-hour 75g OGTT were associated with adverse pregnancy outcomes (large for gestational age, macrosomia, cord c-peptide greater than the 90th centile). In addition, it was clear that maternal and foetal pregnancy risks rose along a continuum as glucose levels increased, with no threshold effect.

Outcome	**Odds Ratio* (95% CI) Fasting glucose**	**Odds Ratio* (95% CI) 1-hour glucose**	**Odds Ratio* (95% CI) 2-hour glucose**
Birth weight > 90th centile	1.38**	1.46**	1.38**
Cord C-peptide >90th centile	1.55**	1.46**	1.37**
Primary Caesarean Section	1.11**	1.10**	1.08**
Clinical neonatal hypoglycaemia	1.08	1.13**	1.10

* Odds Ratio per 1 standard deviation increase in plasma glucose
**statistically significant

Table 1.2. Summary of Hyperglycemia and Pregnancy Outcome (HAPO) study results for primary outcomes.

1.3.3 Evidence for treatment of GDM

Australian Carbohydrate Intolerance Study in Pregnant Women (2005)

The Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS), published in 2005(31), addressed the question of whether treatment of gestational diabetes mellitus improved maternal and fetal outcomes. Of note, women were identified using a selective screening policy. This study randomised women, with glucose values of <7.8 mmol/L fasting and between 7.8 and 11 mmol/L 2 hours after a 75g glucose load, to an intervention group (n= 490) or routine care (n=510) group. The intervention group received obstetrician-led care with physician support, and were offered individualised diet therapy, self-monitoring of blood glucose, with initiation of insulin if target glucose levels (fasting 3.5 to 5.5 mmol/L and 2-hr postprandial values <7 mmol/L) were not achieved. Twenty percent required insulin therapy. The routine care group received standard antenatal care, unless diabetes was suspected, in which case clinically indicated measurement of blood glucose was carried out. Three percent of the routine care group eventually required insulin therapy. These women were not informed of their test results. Interestingly, 1999 World Health Organisation criteria were published approximately the halfway point of the study, and now described any degree of glucose intolerance (such as the levels in the inclusion criteria) as gestational diabetes. Despite this change in nomenclature, the study group elected to continue with the study as planned in the absence of any evidence that treatment of gestational diabetes improved patient outcomes. Participants were not informed of the change in criteria. The primary outcome for infants was a composite of; stillbirth or perinatal death, shoulder dystocia, bone fracture, nerve palsy, admission to the neonatal intensive care unit, and jaundice requiring phototherapy. Secondary outcomes included these, and gestational age, birth weight, and other measures of health. The primary outcomes for mothers were need for induction or Caesarean section. Secondary outcomes included number of antenatal attendances and admissions, weight gain, and pregnancy-induced hypertension.

The intervention group had a significantly lower rate of the infant primary outcome (1% vs. 4%, RR 0.33, p=0.01), although there were more induction of labour (39 vs. 29%, p<0.001) and admissions to the neonatal unit in the intervention group (71 vs. 61%, p=0.01). Birthweight was lower (3.3 vs. 3.5%, p<0.001), and the prevalence of macrosomia (10% vs. 21%, p<0.001) was reduced in the intervention group. In keeping with more intervention into delivery as above, gestational age at delivery was lower in the intervention group. A reduction in pre-eclampsia (12 vs. 18%, p=0.02) was also seen in the intervention group. Of note, there were 5 perinatal deaths (3 stillbirths, 2 perinatal deaths) in the routine care group, but none in the intervention group, although this did not quite reach statistical significance (p=0.08)

For the first time, therefore, a randomised controlled trial had demonstrated that treatment of gestational diabetes impacted on clinically relevant outcomes for mother and baby.

Treatment of mild GDM- Landon et al 2009

The 2009 Maternal-Fetal Medicine Units Network study by Landon et al (32) sought to answer the question of whether treatment of mild degrees of hyperglycaemia in pregnancy was of benefit. 958 women with a glucose value between 7.5 and 11.1 mmol/L one hour after a 50g glucose load, and plasma glucose concentrations meeting National Diabetes Data Group criteria (see table 1.1), but with a fasting glucose level of less than 5.3 mmol were randomised to a treatment (n=485) or control (n=473) group, receiving routine care. The treatment group underwent dietary consultation and therapy, with self-monitoring of blood glucose and insulin if glucose levels were over target (fasting 5.3 or over, or two-hour post-prandial 6.7 mmol/L or over). Nine hundred and thirty-one women with a positive glucose challenge test but normal OGTT, were included with the routine antenatal care group in order to mask the status of the control group to study participants and care providers.

The prespecified primary outcome was a composite of perinatal death, fetal hypoglycaemia, hyperbilirubinaemia, neonatal hyperinsulinaemia (>95th percentile for an unselected obstetric population in the study centres), and birth trauma. Neither the composite outcome (seen in 32.4% and 37% of the treatment and control group respectively) nor individual components of it differed significantly between the groups. However, a number of prespecified secondary outcomes did show an improvement. In the treatment group, the mean birthweight was lower (3.3 vs. 3.4 kg, $p < 0.01$), the neonatal fat mass was lower (427 vs. 464g , $p = 0.003$) , the frequency of large-for-gestational age infants was less (7.1 vs. 14.5%, $p < 0.001$), and infants weighing > 4kg were less common (5.9 vs. 14.3%, $p < 0.001$) . With regard to maternal outcomes, Caesarean delivery was less frequent in the treatment group (26.9 vs. 33.8%, p=0.02) , as was shoulder dystocia (1.5 vs. 4%, p=0.02) and hypertensive disorders of pregnancy (8.6 vs. 13.6%, p=0.01). Therefore, although there was no significant difference between the groups with regard to primary outcomes, Landon et al were able to demonstrate that treatment of milder degrees of gestational diabetes were of benefit to both mother and fetus.

1.3.4 IADPSG Consensus Conference 2010

With the HAPO study results now available, and evidence from both the ACHOIS (31) and Maternal-Fetal Medicine Units Network study (32), suggesting that treatment of even relatively mild degrees of hyperglycaemia in pregnancy improved perinatal outcomes, the IADPSG met to translate these results into clinical practice.

This consensus conference had two major outcomes (33). Firstly, women meeting the cut-off values for diagnosis of diabetes in the non-pregnant adult (Table 1.1) would now fall into the new category of ‘overt diabetes’ rather than GDM. The rationale for this was that this group were felt to be

distinct clinically and biochemically from women with milder degrees of hyperglycaemia. They have an increased risk of congenital anomalies(34), an increased risk of nephropathy and retinopathy during pregnancy(35), and more frequently require insulin treatment to achieve normoglycaemia in a timely manner(36).

Secondly, in the absence of a clear threshold value for glucose levels at which risk of adverse pregnancy outcomes increased, and having considered various cut points, the IADPSG consensus committee ultimately decided to set new values for GDM diagnosis at the mean glucose values for which the odds ratio for adverse pregnancy outcome was 1.75. At an OR of 1.5 (threshold FPG value of 5.0 mmol/L), 25% of the cohort met at least one glucose threshold value for diagnosis, while at an OR of 2.0 (threshold FPG value 5.3 mmol/L), only 8.8% of the cohort met one of the threshold values, raising the concern that many women with a high risk of adverse outcome would be missed. The diagnostic cut-off points chosen lowered the fasting and 1-hr values compared to previous values, while raising the 2-hr value slightly. However, the major change was allowing a diagnosis to be made on just a single abnormal value, a change likely to greatly increase the prevalence of gestational diabetes. (37).

These consensus criteria were published in March 2010, and began to enter clinical use shortly afterwards. At the time of writing, in addition to the IADPSG endorsing the criteria, the ADA(38) and WHO(39) have also endorsed the criteria. However, the American College of Obstetricians and Gynaecologists (ACOG) have not adopted the new criteria, and still recommend a 100g OGTT using the Carpenter-Coustan criteria, for diagnosis, a position endorsed by a National Institute of Health (NIH) Consensus Conference in March 2013(40).

1.3.4 Controversy regarding the IADPSG criteria

Despite the widespread adoption of these criteria, the first GDM diagnostic criteria to be based on perinatal outcomes, debate regarding their

appropriateness continues. Although the relationship between glucose levels during pregnancy and adverse pregnancy outcomes are clear, the absence of a clear biological cut-off above which risk begins to rise has led to much debate about where the diagnostic thresholds should lie. As compared with the older criteria in use (table 1.1), the IADPSG criteria have a lower fasting glucose threshold, a similar one-hour glucose threshold, and a two-hour glucose threshold similar to the Carpenter-Coustan criteria, but higher than the WHO 1999 criteria. Also, in common with the WHO criteria, but as opposed to the Carpenter-Coustan criteria, just a single abnormal value is required for diagnosis. The overall effect of these changes has been to increase the prevalence of GDM significantly. In our own ATLANTIC-DIP cohort, the prevalence increased from 9.4% using the WHO 1999 criteria to 12.4% using the IADPSG criteria (41)(further details of this study follow in Chapter 2). On applying these criteria retrospectively to the HAPO cohort, 17.8% (range 9.3-25.5%) of participants met the criteria for diagnosis of GDM(37). Additionally, cohorts of women drawn from populations of non-white European origin may show a disproportionate rise in GDM prevalence when the IADPSG criteria are applied (42). However, there has been criticism regarding the relative benefit of adopting the new diagnostic criteria when this increased prevalence is considered.

Of course, given the relatively recent introduction of the IADPSG diagnostic criteria into clinical practice, little direct evidence of the impact their introduction may have on pregnancy outcomes, much less long-term follow-up, exists. However, looking at the evidence for treatment of GDM to date, three systematic reviews (two of which included a meta-analysis) have examined both maternal and neonatal outcomes.

Hovarth et al (43) showed that treatment of GDM, compared to standard care resulted in fewer cases of shoulder dystocia (OR 0.40, 95%CI 0.21, 0.75) large for gestational age infants (OR 0.48, 95%CI 0.38, 0.62), and macrosomia (OR 0.38, 95%CI 0.30, 0.49).

Falvignia et al(44) undertook a systematic review of randomised/quasi-randomised controlled trials, and examining outcomes in 3,167 women, showed that GDM treatment resulted in a decrease in macrosomia (RR 0.47,

95%CI 0.34-0.65), large for gestational age (RR 0.57, 95%CI 0.47-0.71), and shoulder dystocia (95% CI 0.41, 95%CI 0.22-0.76), but not in perinatal mortality (RR 0.41 95%CI 0.22-0.76), neonatal ICU admission, or birth trauma. With regard to maternal outcomes, a reduction in pre-eclampsia (RR 0.61, 95%CI 0.46-0.81) and hypertensive disorders (RR 0.64, 95%CI 0.51- 0.81), but not Caesarean section, was shown. A further meta-analysis (45), undertaken for the United States Preventive Services Task Force, showed a reduction in shoulder dystocia (RR 0.42, 95%CI 0.23, 0.77) large for gestational age infants (RR 0.56, 95%CI 0.45, 0.69), and macrosomia (defined as birthweight >4kg, RR 0.50, 95%CI 0.35, 0.71). With regard to maternal outcomes, less preeclampsia (RR 0.66, 95%CI 0.48, 0.90) was seen, and no evidence of harm was seen, although there were more prenatal visits in women treated for GDM.

All of the above studies drew to a large degree on the same pool of studies, and all incorporated results from the ACHOIS and Landon et al studies, among others (mostly using the 100g OGTT for diagnosis). Therefore, taking all of these results into account, it appears clear that treatment of GDM, as compared with standard care, reduces cases of macrosomia (by about half), large for gestational age (by about 40-50%), and shoulder dystocia (by about 60%) , and also results in a reduction in preeclampsia (by about 40%), with no evidence of harm resulting from treatment.

With regard to the later consequences of GDM on the offspring (potentially obesity, metabolic syndrome, and type 2 diabetes), or later consequences for the mother (progression to type 2 diabetes, metabolic syndrome, cardiovascular disease), there is no current evidence to suggest that treatment of GDM during pregnancy reduces the occurrence of either of these sets of outcomes.

As the diagnostic criteria used in the above studies varied, looking at the Landon and ACHOIS studies in particular may provide us with some insight into the potential clinical impact of adopting the new IADPSG criteria. The ACHOIS study(31) randomised women with a similar, albeit slightly lower,

2-hr glucose value post 75g OGTT (7.8 to 11.0 mmol/L), but women with a fasting glucose as high as 7.7 mmol/L could still be randomised to the intervention, or conventional care. Landon et al(32) used a 100g glucose tolerance test, given to women who had glucose of 7.5 to 11.1 mmol/L on a 50g glucose challenge test. Despite not using the 75g OGTT, this cohort enrolled women closest to the proposed IADPSG criteria with regard to incorporating women with relatively mild fasting hyperglycaemia. They randomised women with a fasting glucose of less than 5.3 mmol/L, a one-hour of $\geq$ 10.6 mmol/L, a two-hour of $\geq$ 9.2 mmol/L, and a three-hour of $\geq$ 8.1mmol/L on the OGTT to the intervention or conventional treatment. Therefore, although not directly applicable to the IADPSG criteria, some conclusions (i.e. the expectation that treatment of milder degrees of GDM would show benefit with regard to adverse pregnancy outcome) from these two studies may be considered relevant.

The economic benefit of adopting the new criteria remains uncertain. A single paper proposing three separate models, one of which was the IADPSG-proposed screening protocol, determined that this approach was cost-effective only if post-delivery care (including long-term follow-up) reduced the incidence of diabetes(46). However, this study estimated a cumulative incidence of diabetes of 26% over 15 years, based on the Diabetes Prevention Program (DPP)(47), which was carried out using older diagnostic criteria (and self-reported GDM history); the cumulative incidence of diabetes among women diagnosed using the IADPSG criteria is not yet known.

The IADPSG panel did examine other cut-offs for determining a diagnosis of GDM(48). An OR of 1.5 (corresponding to a fasting glucose threshold of 5.0 mmol/L) would increase the total prevalence of diagnosed GDM in the HAPO cohort (including the 1.7% of women who were unblinded due fasting or 2-hour plasma glucose levels exceeding predefined thresholds of 5.8 mmol/L and 11.1 mmol/L, respectively) to 26.7 %, and was rejected. A threshold of an OR of 2.0 was also considered. This would correspond to a fasting glucose threshold of 5.3 mmol/L and would result in a total GDM

prevalence in the HAPO cohort of 10.5%. The panel felt, however, that the higher threshold values, as compared to those generated when an OR of 1.75 was used, "would fail to identify many cases with a nearly comparable risk of adverse outcomes".

However, despite these criteria being the first set of criteria to be derived based on adverse pregnancy outcome, and representing a major step towards greater homogeneity in GDM diagnostic criteria, there has been much controversy. The IADPSG criteria have drawn criticism on several fronts: the HAPO study, on which the criteria are largely based, was an observational study, and therefore randomised controlled trial evidence of the relative benefits of adopting the new criteria in the treatment of GDM is not available; the increased prevalence of GDM seen with the adoption of the new criteria is likely to have major resource implications, and finally, that adopting a 'glucocentric' approach ignores other factors contributing to large for gestational age infants, and the fact that most large for gestational age infants are born to mothers with normal glucose levels. Ryan (49) argues that increased BMI rather than glucose is responsible for the majority of macrosomic infants (50). Ryan also proposes diagnostic thresholds reflecting an OR of 2.0 for adverse pregnancy outcome using the HAPO data, resulting in a prevalence of 10.5%, as outlined above, on the grounds that diagnosing so many extra women with GDM to prevent a relatively small number of adverse outcomes is of uncertain benefit. This debate continues (51), and at least one randomised controlled trial is planned to compare the impact of using the IADPSG as compared to the Carpenter-Coustan criteria on maternal and foetal outcomes (https://clinicaltrials.gov/ct2/show/NCT02309138). Nevertheless, it is clear that the HAPO study, and the IADPSG recommendations that stem from this, are a major step forward towards the goal of evidence-based, and universal, diagnostic criteria for gestational diabetes. As such, information on the future impact adoption of these criteria will have, including the risk of progression to diabetes later in life in women diagnosed using these new criteria, is essential.

1.4 GDM and subsequent risk of type 2 diabetes

1.4.1 Introduction

With this in mind, we will review what effect changing diagnostic criteria for GDM diagnosis may have on the prevalence of abnormal glucose tolerance/diabetes post-GDM, and what risk factors are associated with progression to diabetes. This is a clinically relevant problem for 2 major reasons- firstly, prevention or delay of type 2 diabetes in women with previous GDM is a possibility, as demonstrated by a subgroup analysis of the Diabetes Prevention Program (DPP)(47), and also in the TRoglitazone In the Prevention Of Diabetes (TRIPOD)(52) and (Pioglitazone In the Prevention Of Diabetes)PIPOD(53) studies. Secondly, undetected type 2 diabetes developing prior to a subsequent pregnancy carries the risk of congenital malformation and an increased risk of pregnancy complications.

Search strategy

Using a combination of the search terms “gestational diabetes”, “diabetes and pregnancy”, “diabetic pregnancy “, “impaired glucose tolerance”, “abnormal glucose tolerance”, “NIDDM”, “non-insulin dependent diabetes”, and “type 2 diabetes”, MEDLINE, OLDMEDLINE, Embase, and the Cochrane Library were searched from 1946 to July 2014, with no language restrictions. Titles and abstracts were reviewed and 376 potentially relevant publications were identified, all of which then underwent full-text review. The reference lists of these articles were hand-searched to identify additional relevant papers. One hundred and thirty-nine papers were included in the final literature review. The following variables were extracted where reported; year of publication, criteria used for GDM diagnosis, screening policy, completeness of follow-up, prevalence of GDM in the population, number of women with GDM in the study, number of women in the control group(s), mean or median age of the women results were tabulated using Excel (Microsoft, Washington) and SPSS (IBM, New York).

Heterogeneity of studied cohorts

Many studies have assessed the risk of progression to type 2 diabetes post gestational diabetes.
A major issue with all studies in this area however, is their marked heterogeneity. This heterogeneity has several aspects;

1) As discussed, the diagnostic criteria in clinical use for GDM diagnosis over the last four decades are numerous. This leads to the identification of cohorts who may not be directly comparable in terms of the severity of glucose intolerance.

2) The method of screening for GDM varies among cohorts- different cohort characteristics may be seen depending on whether or not universal screening, risk-factor based screening, or a 50g glucose challenge test is used.

3) Both the criteria and method used to diagnose diabetes and/or abnormal glucose tolerance in women who have previously had GDM varies significantly.

4) The ethnic mix of the cohorts is extremely heterogeneous with some composed entirely of a single ethnicity, and others showing very mixed composition.

5) Cohort retention at follow-up across studies shows extremely wide variability.

6) Duration of follow-up varies between studies, from 6 weeks to almost 30 years(54).

The heterogeneity of these factors makes a meaningful comparison across the studies in this area impossible. Several reviews have attempted such a

comparison. Kim et al conducted a systematic review on this topic in 2002(54), which clearly demonstrates this heterogeneity. The cumulative incidence of progression to diabetes post gestational diabetes varies from 2.6% to 70%. A subgroup comparison of those cohorts, adjusted for retention rates, and composed only of women with NDDG-defined GDM, compared seven cohorts. This comparison also adjusted for variable follow-up testing rates and for varying lengths of follow-up. This found that the cumulative incidence of diabetes increased markedly in the first 5 years following a pregnancy complicated by GDM, and levelled off at 10 years, although new cases continued to be diagnosed right until the end of the longest follow-up period.

A subsequent meta-analysis by Bellamy et al in 2009 (55)included only cohort studies that included a normoglycaemic control group. The final meta-analysis included 31,867 women with previous GDM, of whom 10,859 developed type 2 diabetes (see forest plot figure 1.4). Heterogeneity was seen when studies were grouped according to the number of cases of diabetes, with smaller studies tending to present larger effects. No effect was seen when studies were grouped according to different diagnostic criteria. Therefore, it can be seen that, although using these stricter inclusion criteria made a meta-analysis possible, heterogeneity across even this limited number of cohorts was evident. For this literature review, in order to get as broad a perspective on possible predictive factors for abnormal glucose tolerance after GDM, we have reviewed the literature from retrospective and prospective cohort studies, and case studies, regardless of whether or not a control group was present, while taking into account the findings from the systematic review and meta-analysis on this topic outlined above.

Therefore, in summary, meaningful comparison of the actual cumulative incidence or prevalence across all of these studies is not possible. It is clear, however, that regardless of the criteria used, GDM signifies a high risk of future progression to type 2 diabetes.

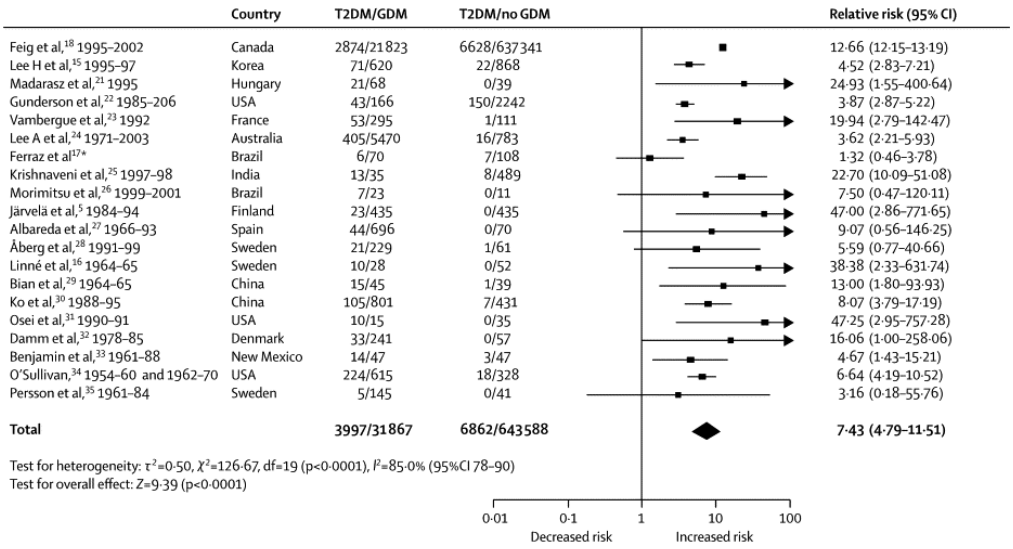

*Figure 1.4 Forest plot demonstrating the risk of type 2 diabetes after gestational diabetes mellitus among twenty case-control studies. Each square represents relative risk for that study, while horizontal lines indicate 95% confidence intervals. Note the marked heterogeneity evident (log scale used on x-axis). Df = degrees of freedom, * = dates not available.*
Reprinted from The Lancet 373 (9677), Bellamy L, Casas JP, Hingorani AD, Willams D. Type 2 diabetes after gestational diabetes: a systematic review and meta-analysis, 1773-9 Copyright 2009, with permission from Elsevier.

1.4.2 Risk factors for future progression

Despite the heterogeneity of the cohorts, many studies identify similar factors predicting progression to diabetes/abnormal glucose tolerance (AGT). We will consider the most commonly associated risk factors here.

Pre-pregnancy factors

Given that most studies identify women with GDM at the time of diagnosis, most studies assess pre-pregnancy risk factors retrospectively. Therefore, information on this is limited. The exception to retrospective recall of pre-pregnancy factors is the large long-term longitudinal cohort population-based studies, such as the Nurse Health Study(56), which have detailed information preceding the index pregnancy. However, these also use self-reported GDM as an outcome measure. Although the diagnosis has been validated in a subset by medical record review, the precise criteria used by the healthcare provider are uncertain, and therefore lie outside the scope of

this review. Of pre-pregnancy variables assessed, weight or BMI is the most common measure, and is commonly associated with increased risk of progression to AGT or diabetes (57-62), although the relationship is not particularly strong. Polycystic ovary syndrome has also been reported in one retrospective study to be associated with later progression to abnormal glucose tolerance on multivariable analysis, although this study used two different sets of criteria to diagnose GDM(63).

Index pregnancy-related factors

Pregnancy glucose values

Higher glucose values during pregnancy, as reflected by the index pregnancy OGTT, are consistently associated with increased later progression to diabetes. This is measured in various ways (number of abnormal values, area under the curve), but most commonly the values for plasma glucose at fasting, one hour, two hours (and three hours if applicable) are used. Fasting glucose shows the strongest association, being the most common risk factor associated with later AGT and diabetes(54, 64-69). Studies that have not identified fasting glucose as a factor associated with later progression to AGT tend to have either not measured it(70), not included it in the statistical models(71), or have excluded women with the highest fasting glucose levels from follow-up. (72, 73) One large Australian study found fasting glucose was not associated with later AGT and diabetes despite its inclusion in the model(74). One-hour(72, 74, 75) and two-hour glucose levels(61, 64, 75, 76) are also associated with later glucose abnormalities, although less consistently, and to varying degrees. Also, higher HbA1c during pregnancy, although much less frequently studied, has been found to be associated with future risk of progression to diabetes (76, 77).

More detailed characterisation of glycaemic response to a glucose load such as measures of insulin secretion(67), when undertaken, are also associated

with later progression to AGT and diabetes. These measures, of course, are generally not available routinely clinically. Insulin use during pregnancy has also frequently been shown to be associated with increased risk of future progression to diabetes/AGT(60, 70, 78-80), presumably as a marker of higher glucose levels in pregnancy, even taking into account likely differences in prescribing practice between centres.

The topic of glucose levels on the pregnancy OGTT that meet the criteria for type 2 diabetes in the non-pregnant patient (i.e. fasting glucose ≥ 7.0 mmol/l or a 2-hour postload glucose value of ≥ 11.1 mmol/L) are controversial. The IADPSG criteria classify this level of dysglycaemia as the new category of 'overt diabetes in pregnancy', suggesting that women meeting these criteria re likely to have had pre-existing unrecognised type 2 diabetes. The evidence to date is limited on later progression to diabetes among the specific category of women meeting these criteria. A single retrospective study to date has looked at this (81), which appears to indicate that the category of overt diabetes in pregnancy cannot be considered synonymous with undiagnosed type 2 diabetes- 41.4% of women meeting these criteria were found to have normal glucose tolerance at early post-partum testing, while a further 37.6% had values indicating abnormal glucose tolerance, but not diabetes. Therefore, although further work will be valuable in refining the risk in these women, it would seem likely that their future risk of progression to diabetes lies along a continuum, rather than showing an abrupt increase in risk consistent with a threshold effect.

Glucose challenge test

The screening policy across individual studies is also an area that could potentially influence the future risk of developing diabetes after GDM, although this information is frequently not reported. Studies may use universal screening with an OGTT (1-step procedure), risk-factor based screening with an OGTT (1-step procedure), or, most commonly among the cohorts for which this information is reported, a 50g glucose challenge test (GCT) followed by an OGTT for those women above a certain threshold (2-

step procedure- also, glucose thresholds for proceeding to OGTT after GCT varies among cohorts). It might be expected that cohorts diagnosed with GDM on the basis of either risk factor-based screening, or following a GCT would represent a higher-risk cohort, who may therefore demonstrate an increased number progressing to later abnormal glucose tolerance. However, the available data does not support this, likely at least in part, due to the heterogeneity among cohorts outlined in the previous section of this chapter limiting comparison across studies. It should be noted, however, that the glucose value itself on the glucose challenge test is independently associated with higher glucose levels postpartum (82, 83).

Completeness of follow-up

Cohort retention, where reported, also varies significantly- from approximately 12% (84) to 100% (85) - across studies, and may be an important factor in the heterogeneity seen among studied cohorts (54). Most studies demonstrate a high proportion of women being lost to follow-up (86). If there are systematic differences between those women who attend and those who do not, this will cause an overestimation of risk if higher-risk women attend, and underestimation of risk if lower-risk women attend for follow-up). In addition, retrospective studies (87) may report only women who have been screened post-partum. This should be borne in mind when extrapolating figures for assessment of risk from any single cohort to a population.

Body weight/BMI

Body weight (or BMI) during the index pregnancy is commonly reported in studies of GDM cohorts, occasionally with waist circumference or body fat measurements. Studies are inconsistent as to whether weight or BMI persist as a risk factor when adjusted for other risk factors using multivariate analysis. Studies that have not found an association between pregnancy weight and BMI tend to examine women who have progressed to AGT in the early post-partum period. BMI during pregnancy may be associated with

AGT at this stage, but is not independently associated when antepartum glucose levels, indicating severity of hyperglycaemia, are included in the model(65). Most studies that do show an association between pregnancy BMI and later AGT involve longer-term follow-up post delivery, (67, 84, 88) although this is not a universal finding.(89)

Gestational age at diagnosis

Gestational age at diagnosis is another commonly reported association (61, 62, 65, 66, 68, 83, 90). However, many of the studies also specify a screening protocol that involves screening higher-risk women in early pregnancy, causing a significant bias. Women diagnosed with GDM in early pregnancy, before insulin resistance begins to rise(91, 92), are likely to have a greater degree of hyperglycaemia, and therefore an increased likelihood of progression to AGT/diabetes. However, gestational age at diagnosis remains a risk factor, even when measures of glycaemia from the index pregnancy are included in the model for many of these studies.(65, 66, 68, 83, 90)

Ethnicity

There are few studies specifically examining the effects of ethnicity, although these that do have generally found an increased prevalence among those women of ethnicity other than white European origin(71, 93-97). Other studies have found no association(64, 98). The reasons for this are unclear. However, many studies have examined ethnically homogenous cohorts, who are often already at high risk of GDM. The prevalence of GDM is higher among ethnic groups who are not of white European origin, while the prevalence of GDM increases at a lower BMI(99) in the Asian populations studied. In addition, adoption of the IADPSG criteria may cause a disproportionate rise in GDM prevalence among Asian populations(42), which will be of relevance when determining the future risk of AGT or diabetes in these populations. In addition to the studies outlined above examining this question, comparison between studies does suggest a higher proportion of women of non-white European ethnicity progress to AGT(97).

However, meaningful comparison between studies is generally not possible due to the heterogeneity of the studies on the points listed above. Of note, Kim et al's systematic review(54), discussed in the previous section, concluded that differences between cohorts could largely be explained by differences in cohort retention and length of follow-up, although this cannot be ascertained directly from the current available evidence.

Family history of diabetes

This is uncommonly associated with progression to AGT/diabetes among women with GDM after measures of glycaemia are taken into account. Several studies examining family history have found no effect (73, 100, 101). Although some studies have shown an independent effect (63, 71, 89, 102), it appears to be small, and the association is often not seen when analysed as part of a multivariate model (62, 84, 103, 104). Therefore, family history does not appear to play a major independent role in predicting future risk of diabetes or AGT.

Other factors

Age at diagnosis of GDM (68, 76, 78, 104) has been associated with future AGT or diabetes also, but is inconsistent, with other studies showing no association (88, 105, 106), and again, is rarely significant(78) when other variables are taken into account. Parity, most commonly classified as a binary variable (multiparous or nulliparous) has been identified (77, 79, 107) as potentially associated with higher risk of later progression, but this finding is inconsistent (65, 106). Potential gene associations have also been identified, but currently appear to add little to clinically assessing individual risk(108). Autoantibody testing also been examined(18), and appears to be associated with risk of progression to type 1 rather than type 2 diabetes. Maturity-onset diabetes of the young (MODY) may also present in pregnancy, although little data on the prevalence among women with GDM is available. Estimates vary between 0-1% for HNF-1α mutations among women with GDM(109)The estimates for the prevalence of a glucokinase

mutation (GCK) among women with GDM vary widely- between 0 and 12%(109) - but has been shown to be 0.1% in our population(19).

Risk factors post-pregnancy

Breastfeeding

Breastfeeding among women with GDM is associated with improved glycaemic indices in the early post-partum period (71, 110). Its role in prevention of later progression to AGT is at present unclear, although long-term follow-up of the SWIFT (Study of Women, Infant Feeding, and Type 2 diabetes mellitus after GDM pregnancy) cohort will address this issue.

Weight/BMI

Weight (or associated measures) after the index pregnancy has been shown to be correlated in a number of studies(57, 80, 89, 111-114) with progression to diabetes or AGT. This correlation appears more robust than that seen with pregnancy weight/BMI, which often loses significance in multivariable models (see above). Also, weight gain since the index pregnancy has been associated with metabolic deterioration(67). Studies not demonstrating BMI as a predictive factor may take high-risk cohorts, for example, entirely composed of participants with postpartum IGT(115), or are carried out in the early post-partum period.(65, 98, 116) Interestingly, Wang et al(112) showed that both waist circumference and body fat performed better than BMI in predicting type 2 diabetes in a Chinese cohort, while Jang demonstrated that waist circumference showed a stronger association than BMI in a Korean cohort.(61) This may help to explain why some Asian cohorts(62, 115) have not demonstrated an association between BMI and future AGT or diabetes, despite longer-term follow-up.

Others

The type of contraceptive- specifically the progesterone-only oral contraceptive -is thought to confer a higher risk(117). Subsequent GDM is also associated with greater risk of progression to diabetes/AGT(111). Age at follow-up is as commonly reported than age at delivery. Although an association with later AGT has been noted, (64, 68, 87, 118, 119), and despite the increasing prevalence of type 2 diabetes with advancing age in the general population, this is not a universal finding(62, 120), particularly in multivariate analysis(121). This may be due to the relatively narrow age range in the cohorts of women involved in these studies, compared to the population as a whole.

Despite the heterogeneity if the studies for the reasons above, including diagnostic criteria used, there is consistency among most studies in the factors associated with a greater risk of diabetes after the index pregnancy in women with GDM. As can be seen, measures of glycaemia during the index pregnancy are not only the strongest predictor, but also frequently attenuate or remove the predictive ability of other traditional risk factors for type 2 diabetes. Thus, the most important risk factor for future AGT or diabetes in these women is simply a previous diagnosis of GDM, taking into account the degree of hyperglycaemia at diagnosis.

1.4.3 Prevalence of diabetes post-GDM

The prevalence of progression from GDM to abnormal glucose or type 2 diabetes varies greatly. The lifetime cumulative incidence of diabetes among women with GDM is frequently cited at up to 60%, but this summary figure does not illustrate the many underlying different factors (e.g. time since delivery, cohort demographics, and criteria for diagnosis of GDM and postpartum diabetes).

Duration of follow-up

With regard to timing, many studies have documented short-term follow-up only (i.e. to the first post-partum test). Prevalence rates at this time point differ, and are generally less than 10%, but depending on the cohort studied, and criteria used, may be significantly higher – Metzger et al showed a prevalence of 38% up to one year post-partum in women meeting NDDG criteria(64). These women are likely to be different from those developing diabetes at a later post-partum interval, and are likely to have had pre-existing type 2 diabetes. It is therefore unlikely that any of the criteria in use for GDM diagnosis would fail to detect these women.

Beyond the post-partum period, prevalence or cumulative incidence figures continue to show great variation. Figures may be as low as 3% (up to 3 years post-partum from a Swedish cohort, using area under the glucose curve measures from the OGTT for diagnosis(113)), and as high as 62% (at up to 6.5 years in a cohort from Trinidad meeting the 1980 WHO criteria(93)). Follow-up of O'Sullivan's original cohort at 16 years showed a cumulative incidence by life-table analysis of 60%(113). As outlined in the previous section, Kim et al's systematic review from 2002 (54) attempted to control for the marked heterogeneity in time among studies, by plotting actuarial projections of cumulative incidence of cohorts at up to 28 years follow-up, and concluded that most cohorts progressed to diabetes at a similar rate in the first 5 years post index pregnancy, and then levelled off by 10 years with few cases after this (this calculation included NDDG-diagnosed studies only).

Cohort features

Cohort selection also plays a vital role in determining later progression to AGT/diabetes, and makes comparison difficult. Selection of women who are known to have normal glucose tolerance in the early post-partum period(72), or restricting follow-up to those who did not require insulin for glycaemic control in pregnancy(58), would be expected to reduce the

proportion progressing to AGT or diabetes, removing those women with the highest glucose levels during pregnancy. Ethnicity, as outlined above, appears also to be a risk factor for progression, with non-white populations demonstrating increased risk, although comparison across studies is difficult.

Criteria used

There is little evidence to directly compare the different criteria in use. One study directly comparing progression in women meeting the NDDG vs. Carpenter-Coustan criteria(106) showed little difference in prevalence of diabetes after GDM (25.5 vs. 25.3%). However, the WHO criteria (Table 1.1) would be expected to show a smaller proportion of women progressing to diabetes/AGT, given the increased number of women identified with GDM compared to the NDDG and Carpenter-Coustan criteria. However, again, direct comparison across studies is difficult. In any given population, therefore, lower diagnostic thresholds will lead to a greater prevalence of GDM. Conversely, criteria using higher thresholds to define GDM will identify fewer women with GDM, but these women will, on average, have higher glucose levels. Therefore, the proportion progressing to AGT/diabetes will be higher, despite the lower GDM prevalence.

Also, the criteria used to diagnose type 2 diabetes and AGT postpartum may differ- older cohorts in particular, using the NDDG or older WHO criteria would be expected to show a lower prevalence at follow-up due to higher thresholds for diagnosis.

1.5 Relevance of IADPSG criteria

The new IADPSG criteria pose an important clinical question with regard to intensity of follow-up. With potentially up to one in four pregnancies in some centres meeting the new criteria for GDM diagnosis(37), lifelong follow-up of these women will have important clinical and resource implications. However, the optimal mode and timing of a follow-up strategy

remains unclear. More women with milder degrees of hyperglycaemia are now classified as GDM. Accordingly, the proportion progressing to AGT should decrease. There are as of yet no prospective figures on progression to type 2 diabetes or AGT post-partum in women with IADPSG-defined GDM. The ATLANTIC-DIP study retrospectively classified women using IADPSG criteria after a universal screening programme, and found that 19% had AGT at early post-partum follow-up(71). Capula et al (63) looked at a mixed (approximately 60% diagnosed by IADPSG criteria) cohort, and found 4% had diabetes, and a further 32% AGT at 6-12 weeks post-partum. Overall, it appears certain that more women will need to be tested to identify those women progressing to AGT and diabetes.

Some clues as to how women diagnosed with GDM by IADPSG criteria may behave on follow-up may be seen in several papers which follow women meeting just a single abnormal value on the pregnancy OGTT using the older criteria. Retnakaran et al(122, 123), using NDDG criteria for GDM diagnosis, examined early post-partum outcomes among women along the spectrum of glucose tolerance: from normal glucose tolerance, to abnormal glucose challenge test (GCT) with normal OGTT, a single abnormal value on OGTT, and GDM. This demonstrated a graded relationship in AGT; from 3.2% in the NGT group, 10.2% in the GCT abnormal, OGTT normal group, 16.5% in the GCT abnormal, single abnormal value on OGTT group, to 32.8% in the GDM group. Indeed, detailed characterisation of these groups(124) demonstrates the similarity between women with a single abnormal value at 1-hr post glucose load (as opposed to later abnormal values) and women with GDM, as measured by AUC curve on OGTT and beta-cell dysfunction at 3 months postpartum.

Thus we can see that a cohort of women with a single abnormal value only, albeit using higher cut-offs than the new IADPSG values, still have a clinically important increased risk of AGT. Other studies examining similar cohorts, although at longer follow-up, have drawn similar conclusions; Steube et al(125), using the stricter Carpenter-Coustan criteria, showed a higher HbA1c in women with a single abnormal value at 3-year follow-up

versus both women with GDM and those with NGT in pregnancy. Carr et al (using Carpenter-Coustan criteria)(126), found a HR of 2.0 for diabetes diagnosis among women with a single abnormal value on OGTT versus those who did not. Vambergue et al (using Carpenter-Coustan criteria)(104) showed a similar graded relationship for progression to type 2 diabetes at almost 7 years follow-up, with 6% of women with a single abnormal value progressing to diabetes, as compared with 18% in the those meeting GDM criteria (less than 1% of those with no abnormal values had progressed to diabetes).

Therefore, all degrees of glucose abnormalities in pregnancy, even those not meeting older GDM criteria are associated with an increased risk of later glucose abnormalities. This may have important implications for those women with lesser degrees of hyperglycaemia who all now be classified as GDM by IADPSG criteria.

Relevance of overt diabetes

Women meeting criteria for diabetes diagnosis in the non-pregnant adult are now classified as separate category by the IADPSG criteria and represent the highest-risk GDM cohort, having an increased risk of congenital abnormalities and diabetes complications, and are likely to have had undiagnosed type 2 diabetes preceding the index pregnancy(33). The future risk of these women is unclear at present, and further prospective follow-up comparing women meeting both sets of IADPSG criteria will be useful in further refining risk in this population, although this is outside the scope of our current study.

1.6 Current post-partum follow-up strategies

Current recommendations for follow-up of women with gestational diabetes vary from region to region. The ADA recommend an early post-partum

OGTT (in line with ACOG guidelines) and follow-up with HbA1c, fasting plasma glucose (FPG) or 75g OGTT thereafter, on a 1-3 yearly basis (127). The International Diabetes Federation (IDF)(128) recommend an early post-partum OGTT, and thereafter vary recommendations on whether a further pregnancy is planned, (OGTT prior to conception) and whether the woman is high-risk (annual OGTT) or low-risk (FPG every 2-3 years), the criteria for which are not defined. The British National Institute for Health and Care Excellence (NICE) guidelines(129) recommend FPG alone in the early post-partum period, and an OGTT at follow-up only if a further pregnancy is planned. This will be particularly important if IADPSG criteria are used, as the optimum frequency and mode of testing for such a large cohort of women with previous GDM is unknown. We will present data on a potential follow-up strategy in chapter 7 of this work.

In summary, the IADPSG criteria are likely to significantly increase the prevalence of diagnosed GDM worldwide. As we have seen above, lower diagnostic thresholds lead to a higher number of women diagnosed, but are likely to lead to a smaller proportion going on to develop AGT.

Therefore, with this work, we have set out to;

1) Determine the prevalence of AGT in women meeting IADPSG criteria for diagnosis up to 5 years after the index pregnancy, as compared with women with normal glucose tolerance (NGT) in pregnancy, and variables associated with AGT after the index pregnancy.
2) Determine the prevalence of metabolic syndrome and insulin resistance in women meeting IADPSG criteria for diagnosis up to 5 years after the index pregnancy, as compared with women with normal glucose tolerance (NGT) in pregnancy.
3) Examine potential methods of follow-up for women meeting IADPSG criteria for diagnosis up to 5 years after the index pregnancy.

Detailed methodology of this study follows in the next chapter.

Chapter 2 – Methods

2.1 Background

2.11 The ATLANTIC-DIP programme

The ATLANTIC-DIP (Diabetes in Pregnancy) programme commenced in 2006 in the Health Service Executive (HSE) West region. The geographical region included in the ATLANTIC-DIP project extends from the North-Western region of Ireland (Co. Donegal) down to Galway city in the South (Fig 2.1), covering an area of 7338 square miles, and encompassing a population of approximately 500,000 people. There are approximately 11,000 births per annum in this region(130). This area contains five hospitals that provide an antenatal service to women, including those with diabetes, at Galway Roscommon University Hospitals Group centres, located in Galway city and Ballinasloe , Co Galway; Mayo General Hospital, Castlebar, Co Mayo; Sligo General Hospital, Sligo, Co Sligo; and Letterkenny General Hospital, Letterkenny, Co Donegal. Of note, Galway is a tertiary referral centre for women in this region, and has full neonatal intensive care facilities (with the exception of facilities for therapeutic hypothermia). The original study was funded by a grant from the Irish Health Research Board (HRB) and ran from January 2006 to June 2011. This involved three separate arms with different aims. The first part of the ATLANTIC-DIP study involved the prospective collection and analysis of pregnancy outcomes (maternal and infant) among women with pregestational diabetes (Type 1 or Type 2) diagnosed at least 6 months prior to the index pregnancy and attending for antenatal care in one of the five hospitals sites in the region(131). The second part of the ATLANTIC-DIP study was designed to establish the true prevalence of GDM in Irish women using universal screening. The remaining arm of ATLANTIC-DIP examined the pregnancy outcomes for women with GDM diagnosed through universal screening and their babies compared to women known to have normal glucose tolerance (NGT) in pregnancy. (41). Local research

ethics committee approval was obtained prior to the commencement of the programme of work (University Hospital Galway ethics committee). Universal screening was carried out using a one-step process- a 75g oral glucose tolerance test (OGTT), with fasting, one-hour, and two-hour plasma glucose levels was used. For the purposes of this study, women were assigned to a centre on the basis of where their OGTT during pregnancy was performed. This meant that if women in one of the participating centres were later to referred to the tertiary facility (University Hospital Galway), their assignment for this study remained as the original centre in which the OGTT was performed. All women without a previous diagnosis of type 1 or type 2 diabetes booking for antenatal care in the five centres were invited to participate by undergoing a 75g OGTT between 24 and 28 weeks gestation. WHO criteria (Table 1.1) for the diagnosis of GDM/impaired glucose tolerance (IGT) in pregnancy were employed in accordance with clinical practice at the time of the study. At the initial visit, all women, in addition to the 75g OGTT, had simple demographic information- age, place of residence, ethnicity- recorded, in addition to weight, height and blood pressure measurements. Those women who were diagnosed with GDM/IGT were seen in the combined obstetric/diabetes antenatal clinic held at each site on a two-weekly basis following diagnosis. These women had weight, blood pressure, and urinalysis recorded at each visit in accordance with standard clinical practice. Women with a diagnosis of GDM were treated with medical nutritional therapy (MNT) and exercise advice in the first instance, and also instructed in self-monitoring of blood glucose (SMBG) 7 times per day - fasting, pre-and one hour post-meals, and at bedtime. If SMBG targets remained out of the desired range of <5.5 mmol/L pre-meals and fasting and <7.8 mmol/L 1 hour post-prandially insulin therapy was instituted. Glycaemic control was assessed using SMBG and haemoglobin A1c (HbA1C) measurements. Pregnancies were followed to term and information on obstetrical complications (pregnancy loss, pregnancy-induced hypertension, polyhydramnios, shoulder dystocia, mode of delivery), perinatal outcomes (congenital malformation, stillbirth, birthweight, neonatal death) and post-partum information on infant outcome and breastfeeding was recorded.

2.1.2 ATLANTIC-DIP universal screening component

Using the inclusion criteria above, 12,487 women were invited to participate in the study. Of these women, 3,237 (25.9%) declined the invitation. 9,242 women consented to participate (74.1%). Of those consented, 3,742 (30%) did not attend a scheduled OGTT appointment. This left a cohort of 5,500 women (44%) who completed the study(41).

The study found a prevalence of GDM of 9.4% (n=520) using the WHO criteria. However, when the newer International Association of Diabetes in Pregnancy Study Groups (IADPSG) criteria were applied, the prevalence increased to 12.4% (n=680, see Table 1.1)

2.1.3 ATLANTIC-DIP-post-partum testing

Following this initial screening study, a further arm aimed to evaluate the prevalence of post-partum pre-diabetes and diabetes following GDM. All women meeting IADPSG criteria for diagnosis of GDM during the course of the universal screening component of the programme (n=323) were invited to attend for a 75g OGTT at 12 week post –partum, of whom 300 (92.9%) accepted the invitation and underwent a repeat OGTT. For the purposes of comparison, a cohort of 264 women with documented normal glucose tolerance (NGT) during the universal screening period also underwent retesting. They were classified into either normal (fasting glucose <5.6 mmol/L, 2 hour glucose < 7.8 mmol/L) or AGT. Abnormal glucose was further subdivided into four categories based on the Irish Health Service Executive national guidelines(132), which use the American Diabetes Association (ADA) criteria(38); 1) impaired fasting glucose (IFG) – fasting glucose ≥ 5.6 mmol/l, < 7.0 mmol/L and 2 hour glucose <7.8 mmol/L; 2) impaired glucose tolerance (IGT) – fasting glucose < 5.6 mmol/L, 2 hour glucose ≥ 7.8 mmol/L, <11.1 mmol/L; 3) combined IFG and IGT; 4) diabetes mellitus (DM) - fasting glucose ≥ 7.0 mmol/L, 2 hour glucose ≥ 11.1 mmol/L, or both (Table 2.1).

This arm of the study demonstrated a prevalence of AGT (AGT- all categories) of 19% in the group with GDM versus 2.7% in the group with NGT during pregnancy(71). This study also examined index pregnancy factors associated with progression to AGT post-partum. Factors identified on multivariate analysis (aOR= adjusted odds ratio, CI= confidence interval) were; ethnicity (aOR 3.4 for women of non-European descent, 95% CI 1.45, 8.02), insulin use during pregnancy (aOR 2.62, 95% CI 1.17, 5.87), family history of diabetes (aOR 2.14, 95% CI 1.06, 4.32), elevated BMI at weeks 24-28 gestation (aOR 1.08, 95% CI 1.03, 1.14), and breastfeeding, which was found to be protective (aOR 0.418, 95% CI 0.20, 0.89).

It was on this background that that the current study was conceived, the primary outcome of which was to determine the prevalence of AGT up to five years post partum in the same cohort of women (see Appendix 1- Study Protocol).

Plasma glucose (mmol/l)	**Normal glucose tolerance (NGT)**	**Impaired fasting glucose (IFG)**	**Impaired glucose tolerance (IGT)**	**Combined IFG/IGT**	**Diabetes Mellitus (DM)**
Fasting (FPG)	≤ 5.5	5.6-6.9	≤ 5.5	5.6-6.9 **AND**	≥7.0 **OR**
2 hour value (2hPP)	≤7.7	≤7.7	7.8- 11.0	7.8-11.0	≥11.1

Table 2.1 American Diabetes Association 75g oral glucose tolerance test (OGTT) interpretation

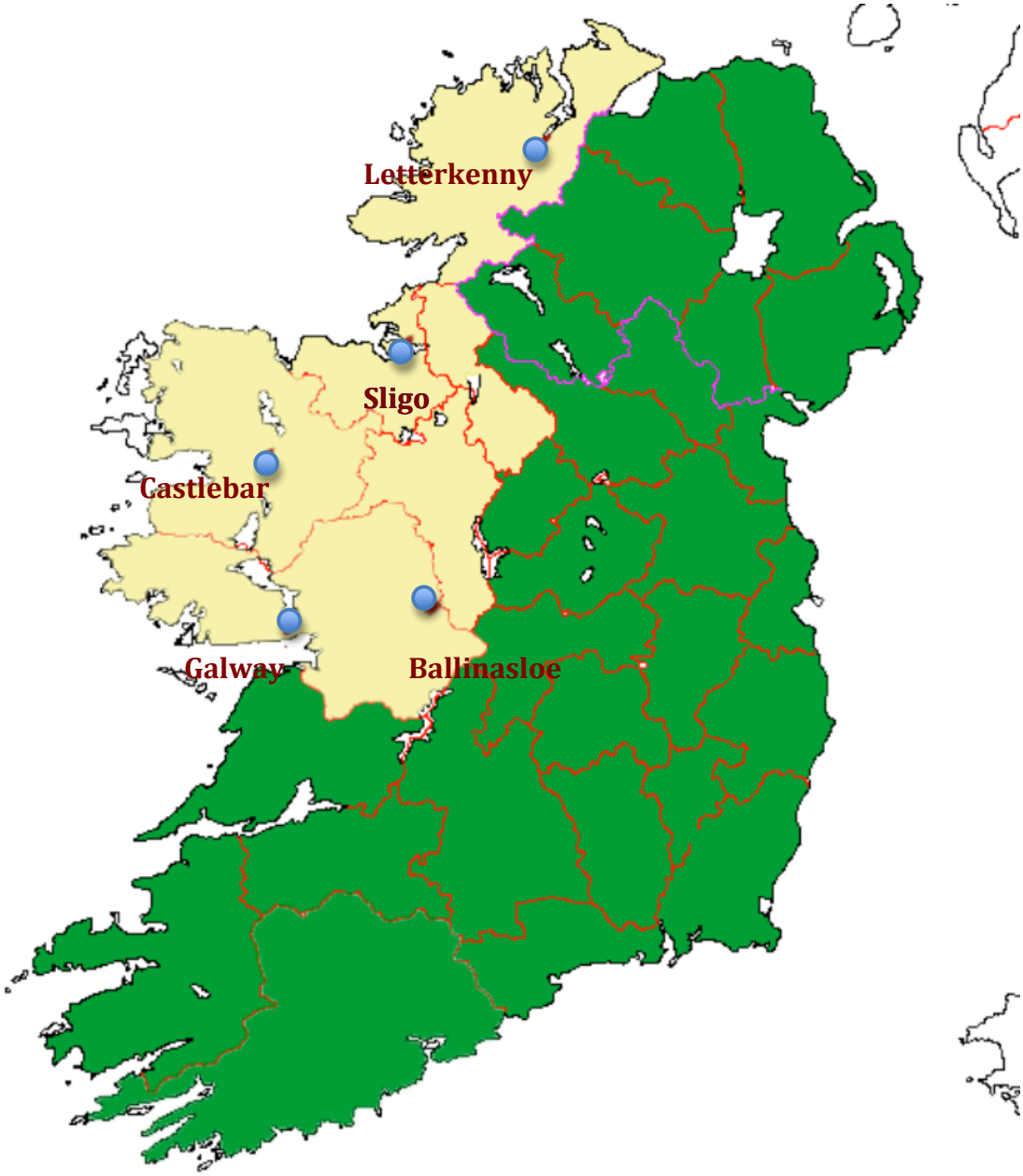

Figure 2.2 Area covered by ATLANTIC-DIP study. Study centres highlighted.

2.2 DIAMOND database

All clinical and laboratory data obtained in the evaluation of the women from the ATLANTIC-DIP cohort was entered in real time into a commercially available database to facilitate clinical care and audit. Following informed consent from each participant, demographic details, clinical details and laboratory results were entered into the DIAMOND® database.

The DIAMOND database is a widely used tool in the clinical management of diabetes throughout Ireland and the United Kingdom, and has been developed and commercialised by Hicom (Woking, UK), a company specialising in the provision of healthcare information technology services. DIAMOND is a Microsoft (Redmond, Washington) Access®-based database, which may be accessed by institutionally authorised users only. It contains a combination of numeric and free text fields, supplemented by drop-down menu options for commonly used fields (e.g. types of insulin prescribed). The participant's full demographic details – name, contact details, date of birth, place of residence, ethnicity, general practitioner name and address, are entered on first attending our service. At their first clinical encounter, a medical and full obstetric history is taken. These are entered into the database. Relevant details arising from their assessment are then entered into pre-defined fields, for example, blood pressure measurement, dipstick urinalysis results, or changes made to medication. Laboratory results obtained at that visit are entered directly at a later date, or, in some cases, transfer directly once the result is available on the Hospital Information System (HIS) server.

This data, as well as providing a useful background for the clinical care of the patient, also serves as an invaluable research and audit tool. The audit function built into the DIAMOND system enables focused searches on numerous parameters. For example, for this study, the audit tool was used to create a list of women diagnosed with GDM on the basis of IADPSG

criteria between 2006 and 2010, who received their care in Galway Roscommon University Hospitals. The audit function, with regard to this study, therefore enabled us to create a database of the patients specified, with their contact details, place of residence, and previous care within our service.

2.3 Study design

2.3.1 Participants

This study was conceived as a single-arm prospective cohort study to determine the prevalence of AGT, metabolic syndrome, and insulin resistance in a group of women of European ancestry up to 5 years post-pregnancy complicated by GDM. For the purposes of this study, as outlined above, we identified a population of women who had participated in the original study whom we wished to re-test up to 5 years post the index GDM pregnancy. Our first step in this process was to create a database of women who met our inclusion criteria for participation in this study (see above).

Using the audit tool in the DIAMOND® database, we selected women from Galway Roscommon University Hospitals Group at Galway and Ballinasloe sites; Mayo General Hospital, Castlebar and Letterkenny General Hospital, Donegal, who were screened during the universal screening component of the ATLANTIC-DIP and who met IADPSG criteria(33) for diagnosis of GDM. This was determined on the basis of their 75g oral glucose tolerance test during pregnancy (fasting glucose ≥ 5.1 mmol/L, one hour glucose ≥ 10.0 mmol/L, two hour glucose ≥ 8.5 mmol/L). For practical and economic reasons, Sligo General Hospital was not included in this study.

In order to best characterise the progression from GDM to impaired fasting glucose/impaired glucose tolerance (IFG/IGT) and type 2 diabetes in our population, we elected to retest those women of European ancestry only. We decided on this approach for several reasons. Firstly, this cohort of women accounted for 83.3% of women tested during the universal

screening component of the ATLANTIC-DIP study(41). Secondly, the effect of ethnicity on the incidence of type 2 diabetes has been well documented(133), but due to significant heterogeneity in the ethnic composition of most studies to date, the precise effect of a previous diagnosis of GDM using IADPSG criteria on a white European population is not clear.

Having identified a cohort of women meeting our pre-specified inclusion criteria, we prepared a database in spreadsheet format (Excel ®, Microsoft, Redmond, Washington) of eligible women from our DIAMOND system. Using this database, we sent each woman thus identified an invitation letter to participate in the study, and a patient information sheet detailing the purpose of the study and the study procedure. Contact details to arrange a screening appointment were provided on the letter mailed to each participant. Following the mailing of letters, each participant who did not respond was contacted by telephone, and again invited to attend, and any questions regarding the study were addressed at this stage. An appointment was scheduled for those women who agreed to participate. If a woman failed to attend for a scheduled appointment, they were contacted via telephone and invited to re-attend for a new appointment.

A database was also created for the control group - women who had participated in the universal screening arm of the previous study, but were found to have NGT on their 75g OGTT during this time (i.e. did not meet IADPSG criteria for diagnosis of GDM), and who met the other inclusion criteria as previously specified. Due to the large number of women meeting these criteria, in the main study centre (University Hospital Galway) we selected controls randomly from this population, using a commercially available random sequence generator(134). All women with NGT from the other three sites were invited to attend if inclusion criteria (see below) were met. Those women selected were contacted and invited to participate by letter initially, and subsequently by follow –up telephone contact.

2.3.2 Study aims

This study was conceived as a single-arm prospective cohort study to determine the prevalence of AGT in a group of women of European ancestry up to 5 years post-pregnancy complicated by GDM. As outlined above, all women diagnosed with GDM using IADPSG criteria between 2006 and 2010 were eligible for inclusion in the study. Women who were currently pregnant, of non-European ancestry, or who had a clinical diagnosis of type 1 diabetes, following the index pregnancy were not eligible for inclusion in the study. A control group of women who were known to have NGT during the ATLANTIC-DIP screening program were also enrolled.

The pre-specified primary outcome (Appendix 1-study protocol) was the prevalence of AGT in a cohort of women with a history of GDM up to five years post index pregnancy, as compared to a cohort of women with NGT in pregnancy during the same time. Pre-specified secondary outcomes were; the prevalence of metabolic syndrome by Adult Treatment Panel III criteria; the prevalence of insulin resistance (IR) defined for this study by a HOMA2-IR value greater than the 90^{th} percentile of the control group; and the prevalence of overweight (BMI 25.0-29.9 kg/m^2) and obesity (BMI 30 kg/m^2 or greater) up to 5 years post partum. Using the information obtained in this study, our objectives were to identify risk factors associated with progression to AGT post GDM, and to make recommendations for a pragmatic, effective rescreen and recall program at a national level.

2.3.3 Data Collection

On attending for screening, informed consent was obtained from each woman (See Appendix 2- Patient information leaflet). Each woman who attended had been advised to fast for 8-12 hours prior to attending for screening. Participants were not advised regarding any carbohydrate restriction for the days preceding the test. Women were also instructed to

take their usual medication in the days leading up to the test. As the numbers on antihyperglycaemic therapy (n=4), and antihypertensive therapy (n=12) were small, and unlikely to influence the overall results for the cohort, no further adjustment was taken for the effect of treatment in the analysis. Blood samples were drawn for fasting glucose, fasting insulin, fasting C-peptide, haemoglobin A1c, vitamin D, and a lipid profile (total cholesterol, HDL-cholesterol, LDL- cholesterol and triglycerides).

Each participant was then given a 75g glucose load (113 mls of Polycal ®, made up to 200mls with water), and instructed not to eat or drink anything further for the duration of the test. Patients were also instructed not to smoke. A detailed questionnaire was completed by the healthcare practitioner, who discussed these questions with the participant (see Appendix 3- Data collection sheet). This contained questions on cardiovascular risk factors, diabetes risk factors, previous obstetrical history, breastfeeding data, and socioeconomic factors. Simple anthropometric measurements were then performed, including waist circumference and neck circumference.

Waist circumference was measured to the nearest 0.5cm, midway between the inferior margin of the last rib and the iliac crest, in the mid-axillary line(135). Neck circumference was measured at the midway point between the mid cervical spine and mid anterior neck, at mid-neck height, to the nearest 0.5cm, with the head placed in the neutral position, and shoulders relaxed(136). A narrow, flexible inelastic tape measure was used for these measurements. Weight was measured to the nearest 100g in light clothing, without shoes, using a calibrated digital weighing scales (Seca ® 635 flat scales). Height was measured to the nearest 0.5cm (Seca ® 635 flat scales). Blood pressure was taken twice, five minutes apart, with the patient sitting comfortably, using an appropriately sized arm cuff, and a calibrated automated blood pressure monitor on the right arm (Philips M3 physiological monitor). The mean of the two measurements obtained (in mmHg) was recorded. Two hours after the initial samples were taken, compliance with the test criteria was once again checked (no

eating/drinking/smoking, no strenuous exercise), and a further glucose sample was obtained. Two trained healthcare professionals carried out the clinical measurements. No systematic differences were evident when these data were analysed by date and site (indicating which healthcare professional carried out the testing). No coefficient of variation was obtained on the measurements.

2.4 Laboratory measurements

All laboratory measurements were carried out at the primary study site (University Hospital Galway). Samples from other sites were separated and frozen (-20 degrees Celsius, 4 degrees Celsius for HbA1c) on-site for transfer (at -20 degrees Celsius) to University Hospital Galway.

2.4.1 Glucose

Plasma glucose measurements were carried out using the hexokinase method, a recognised reference method for the determination of glucose concentration. Hexokinase catalyses the phosphorylation of glucose to glucose-6-phosphate. This is in turn oxidized, in the presence of NADP, to gluconate-6-phosphate, by glucose-6-phosphate dehydrogenase. NADPH is formed during this reaction, and can be measured photometrically at 340nm (ultraviolet region). The rate of formation of NADPH is directly proportional to the glucose concentration. This was carried out using the Roche Modular Analytics P Chemistry Systems. Glucose was collected into flouride oxalate tubes, and analysed within 4 hours of sample collection. Flouride oxalate prevents degradation of glucose, although a decrease in measured glucose concentration of up to 0.6 mmol/L is well-described due to glycolosis in the first 30 mins after collection, which is not prevented by flouride oxalate (137). Between-run coefficient of variation (CV) for glucose at mean concentrations of 2.56 mmol/L, 7.11 mmol/L and 16.7 mmol/L were 1.1%, 0.9 % and 1.0%, respectively.

2.4.2 Haemoglobin A1c

Haemoglobin A1c estimation was carried out on whole blood, anticoagulated with ethylenediaminetetraacetic acid (EDTA). This was carried out using an automated (Menarini HA8160) analyser. After enzymatic cleavage, yielding glycated (HbA1c) and non-glycated (HbAo) N-terminal ß-chain hexapeptides, the peptide mixture is analysed. The haemoglobin fractions pass via a High Pressure Liquid Chromatography (HLPC) column containing microparticles of a copolymer of methylacrylate ester. Haemoglobin fractions are retarded to varying degrees dependent on their molecular size and electrostatic attraction to particles. Sequential washing with solutions of different ionic strengths releases the various haemoglobin fractions in turn, which then pass through an optical flow cell. Absorbance at 415nm and 500nm is recorded on a chromatogram, and the area under the 'peaks' thus recorded is calculated and expressed as HbA1c concentration in mmol/mol of haemoglobin (International Federation of Clinical Chemistry- IFCC - units). The IFCC-DCCT/NGSP master equation (NGSP= 0.09418*IFCC [mmol/mol] + 2.152) was used to derive a DCCT (Diabetes Control and Complications Trial) measurement, expressed as a percentage, in accordance with the NGSP (National Glycohaemoglobin Standardisation Program(138)). This conversion was performed to enable comparison both with the same participants' measurements during their index pregnancy, and with the established body of literature on the subject.

2.4.3 Total Cholesterol

Total cholesterol was analysed using the enzymatic colorimetric technique. Cholesterol is desterified by cholesterol esterase into free cholesterol and fatty acids. Optimisation of cleavage (>99.5%) at this stage allows standardisation and a direct comparison with CDC (Centre for Disease Control and Prevention) and NIST (National Institute of Standards and Technology) reference methods (139-141).

The free cholesterol is then exposed to cholesterol oxidase, yielding 4-

cholesten-3-one and hydrogen peroxide. This reaction is coupled to a chromogen - the hydrogen peroxide reacts with 4-aminophenazone and phenol, catalysed by peroxidase, producing a red coloured product. The intensity of this can be measured photometrically, and is directly proportional to the concentration of cholesterol. The Roche Modular Analytics P Chemistry Systems assay was used in this study.

2.4.4 Triglycerides

Serum triglycerides were also determined using the enzymatic colorimetric technique(142). Microorganism – derived lipoprotein lipase is used to hydrolyse triglycerides to fatty acids and glycerol. The glycerol is then phosphorylated to glycerol-3-phosphate by glycerol kinase. This is then oxidized by glycerol oxidase, forming dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide then reacts with aminophenazone and 4-chlorophenol to form a chromogen (Trinder endpoint reaction). Again, the intensity of this can be measured photometrically, and is directly proportional to the concentration of cholesterol(143). This is carried out using the automated Roche Modular Analytics P Chemistry Systems.

2.4.5 HDL-cholesterol

The Roche Modular Analytics P Chemistry System was used to measure serum HDL concentration, and meets the 1998 National Institutes of Health/National Cholesterol Education Program (NIH/NCEP) standards for acceptable performance. Cholesterol esterase and cholesterol oxidase are modified by PEG. (polyethylene glycol). They then show selective catalytic activity towards lipoprotein fractions (in the order LDL < VLDL < chylomicrons < HDL). Cholesterol esters are broken down by the modified cholesterol esterase into free cholesterol and fatty acids. Free cholesterol is then oxidised to cholestenone and hydrogen peroxide by cholesterol oxidase. Peroxidase then catalyses the reaction of hydrogen peroxide with 4-amino-antiyrine and sodium N-(2-hydroxy-3-sulfopropyl)-3,5-

dimethoxyaniline to form a purple-blue dye, the intensity of which can be measured photometrically and is directly proportional to the cholesterol concentration. The results obtained using this method correlates with those obtained by both precipitation and ultracentrifugation methods.(144).

2.4.6 LDL-cholesterol

LDL cholesterol was not measured directly. In accordance with standard laboratory practice in our centre, the Friedwald equation(145) (below) was employed to estimate LDL-C concentration.

LDL-cholesterol (mmol/l)= Total Cholesterol- HDL-cholesterol – triglycerides/2.2

The triglyceride /2.2 portion of the equation provides an estimate of the VLDL concentration. This equation is not valid when triglycerides are greater than 4.5 mmol/L, due to the alteration of the typical ratio of triglycerides to VLDL at these levels. Therefore, LDL-cholesterol values were not calculated in these patients.

2.4.7 Insulin

Insulin levels were measured using the Roche E170 Modular Analytics Immunoassay System. This is an automated two-site non-competitive immunochemiluminescent assay. Two insulin-specific monoclonal antibodies are used, each directed at different epitopes of the insulin molecule. In the first step, the serum is incubated with both a biotinylated monoclonal anti-insulin antibody, and an antibody labelled with an electrochemiluminescent ruthenium complex. Together, these react to form a sandwich complex. Streptavidin-coated microparticles are then added. This complex then binds to the solid phase due to the interaction of biotin and streptavidin. In the second step, the reaction mixture is aspirated into the measuring cell, where the microparticles are magnetically captured onto the surface of the electrode, and unbound substances removed with ProCell,

a phosphate tripropylamine buffer. Voltage is then applied to the electrode, inducing chemiluminiscent emission, which is measured by a photomultiplier, which converts photons to an electric signal. Results are determined via a calibration curve, which is instrument-specific (generated by two-point calibration and a master curve provided). Insulin was collected into gel tubs and separated and frozen within 2 hour of collection. Thawing and refreezing was avoided entirely, and analysis was carried within 48 hours of collection. The between-run CVs for insulin at mean concentrations of 127 pmol/L, 367 pmol/L and 880 pmol/L were 1.8%, 1.9 % and 1.1%, respectively.

2.4.8 C-peptide

C-peptide levels were analysed using the Roche E170 Modular Analytics Immunoassay System. This is a two-site immunometric assay. Patient serum is incubated with a biotinylated monoclonal C-peptide specific antibody and a ruthenium – labelled monoclonal C-peptide specific antibody. These form a complex, which binds to the solid phase (Streptavidin –coated microparticles). This complex is magnetically captured on the electrode surface, and when voltage is applied to the electrodes, chemiluminiscent emission ensues. This emission, detected by a photomultiplier, is measured against a calibration curve to yield a final result. Insulin was collected into gel tubs and separated and frozen within 2 hour of collection. Thawing and refreezing was avoided entirely, and analysis was carried within 48 hours of collection. The between-run CVs for C-peptide, at mean concentrations of 398 pmol/L, 1310 pmol/L, and 2750 pmol/L were 1.3%, 1.2 % and 1.3%, respectively.

2.5 Statistical analysis

2.5.1 Software packages

Data collected for the study was entered into the DIAMOND clinical

information system, and analysed using SPSS (Statistical Package for the Social Sciences- IBM, New York) version 20, R (R Foundation for Statistical Computing, Vienna) version 18.0, Minitab version 15 (Minitab Inc., Pennsylvania), and a scientific Python 2.7 stack (SciPy http://www.scipy.org, including scikit-learn 0.14 and statsmodels libraries 0.5). Data collection forms are shown in Appendix 3. Statistical significance was set at $p<0.05$ throughout the study. Both p values and 95% confidence intervals, where applicable, are shown throughout this work in order to characterise data as completely as possible.

2.5.2 Sample size

As outlined above, the cohort of women with a diagnosis of GDM rescreened from the original ATLANTIC-DIP universal screening study showed a 19% prevalence of AGT at 12 weeks post partum. A control group of women with NGT during pregnancy demonstrated a 2.7% prevalence of AGT at 12 weeks post-partum. We estimated that a sample size of 88 in each group would give power of 80% to detect a 20% absolute difference in the prevalence of AGT between each group.

2.5.3. Descriptive statistics

All data collected as shown on the study form (shown in Appendix 3) was entered directly into a database created in SPSS version 20. As patients' laboratory results became available, these were entered into the database, and 'dummy' variables, used to create binary outcomes from continuous and categorical variables were employed as necessary. These figures were then used to create variables for scoring systems used in the study, namely; presence of metabolic syndrome (ATP III criteria(146)), HOMA2-IR insulin resistance score(147) (see chapter 4), FINDRISC score (148)(see chapter 5), and Body Mass Index (BMI).

For categorical variables (see table 3.1), the Pearson chi-squared test was used to estimate the difference between population proportions, expressed

throughout this text as percentages. For continuous variables (see Table 3.1), Student's t-test was used to estimate the difference in means between groups, (parametric test) and for continuous variables without a normal distribution, the Mann-Whitney U test was used to estimate differences between the groups (non-parametric test). Central tendency of continuous variables was expressed as the mean and standard deviation for normally distributed data, and as the median, with the observed range of values, for data without a normal distribution. The Bonnet-Price test was used to estimate the confidence intervals between medians where appropriate. For subgroup analysis where more than two groups were created, ANOVA (one-way analysis of variance) was used for parametric testing, while the Kruskal-Wallis one-way analysis of variance was used for non-parametric testing.

To examine correlations between variables, Pearson's correlation coefficient was calculated for normally distributed data, while Spearman's rank correlation coefficient was used for those variables that did not show a normal distribution.

2.5.4 Predictive modelling

To determine which variables were significant predictors of the primary outcome (presence or absence of AGT up to five years post index pregnancy), binary logistic regression analysis was employed. Given the significant co-linearity between variables from the index and variables at rescreening, we constructed two separate logistic regression models to examine the effects of 1) index pregnancy factors associated with progression, and 2) the first model, but with factors between the index pregnancy and restesting added, associated with progression to AGT. Variables associated with the primary outcome on univariate analysis, or with information gain of 5% or more on decision tree analysis (see below) were included in the model. These were the multivariate models used to examine associations between variables and future AGT. All ORs are therefore expressed as adjusted odds ratios (aOR). The relationship of each

variable to the outcome of interest was expressed as the adjusted odds ratio (aOR) for each individual variable, and rounded to one decimal place. The relative contribution of the variables in the model to the observed variation was estimated using the pseudo r^2, larger values indicating greater relative contribution.

We also employed decision tree analysis to determine which variables were associated with future progression to AGT. This used a technique known as classification and regression tree analysis (CART). This technique uses binary splitting (yes or no) on the entered clinical variables to create ('grow') a decision tree(149). It splits the variables using a technique called gini impurity. This is a measure of how homogenous or 'pure' the data presented are on a given variable. It then tries to split them to achieve the best possible separation between the groups with regard to the likelihood of the chosen outcome (i.e. high vs. low-risk). The gini value itself is between 0 and 1, with 0 being the purest, and 1 being the most pure (i.e. most heterogeneous data). Multidimensional scaling (MDS), a technique that uses uses a non-linear mapping to transform measurements in a high-dimensional space (all continuous variables available in the study) down to 2 dimensions for simple visualization was also used to graphically represent our ability to discriminate between women progressing to AGT and those with sustained NGT. Finally, in order to characterise fully the relationship between the variables measured and future glycaemic abnormalities, we carried out multivariable linear regression analysis (ordinary least squares method) with fasting glucose and HbA1c at retesting as outcome variables.

The results obtained in this manner are described in detail in the following chapters.

Chapter 3- Prevalence of abnormal glucose tolerance up to 5 years post-gestational diabetes mellitus

3.1 Introduction

As outlined in chapter 1, the IADPSG guidelines for GDM employ lower diagnostic thresholds in order to make the diagnosis of GDM. Therefore, we would expect as the prevalence of GDM rises, the proportion of women progressing to AGT will fall. However, accurately determining the precise risk of these women, and the factors associated with higher future risk of AGT is vital. This information is clinically relevant when designing and implementing an effective and practical follow-up system. The Diabetes Prevention Program showed that the onset of diabetes could be delayed in women with impaired glucose tolerance and a previous history of GDM, using lifestyle intervention or metformin treatment(47). Secondly, diagnosis of diabetes in women with previous GDM will help to avoid the potential foetal implications(34) of undiagnosed type 2 diabetes presenting for the first time in pregnancy.

Using IADPSG criteria, our study group has shown a 12.4% prevalence of GDM, employing universal screening, as compared with 9.4% using the 1999 WHO criteria. 19% of women with IADPSG-defined GDM demonstrated AGT at early post-partum testing (up to 6 months after delivery)(150). For the current study, these women were invited for retesting up to 5 years post the index GDM pregnancy. Our primary objective was to determine the prevalence of AGT (AGT) up to 5 years post-partum in a cohort of women meeting IADPSG criteria for GDM. Our secondary aims were to; identify which routinely available clinical variables are useful in predicting which women are more likely to progress to AGT post-partum; and finally, to determine factors associated with different measures of glycaemia (fasting plasma glucose- FPG- and HbA1c) up to 5 years post-partum.

3.2 Results

3.2.1 Study participants

We identified 4405 potentially eligible participants (491 meeting IADPSG criteria for GDM, 3914 with NGT) from the 5500 women who participated in the original ATLANTIC-DIP study (Fig. 3.1).

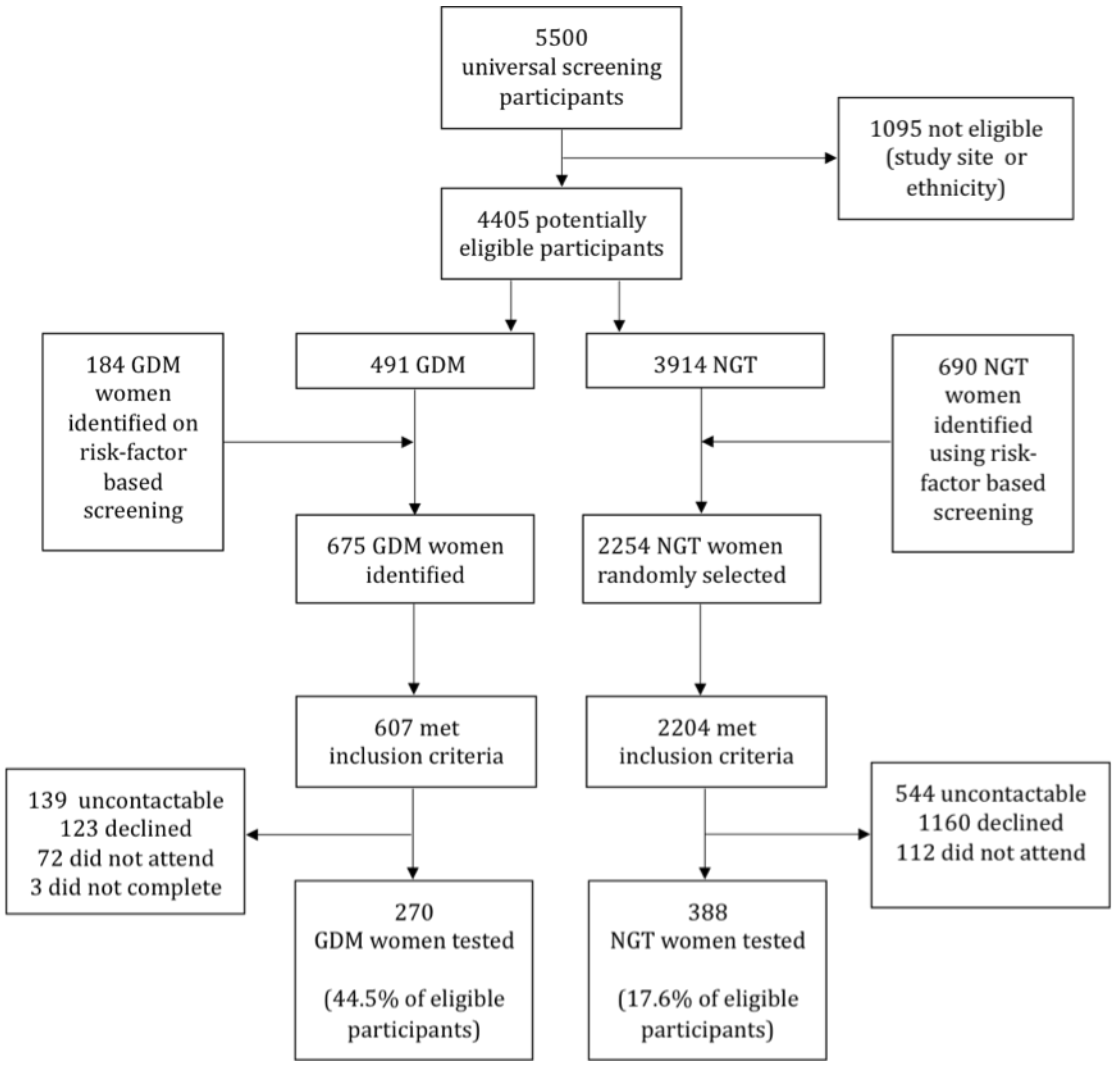

Fig 3.1. Flow diagram for study participants

In addition to this, we identified 874 (184 with IADPSG-defined GDM, 690 with NGT) who were tested using risk-factor based screening. These

women were tested outside of the timeframe of the ATLANTIC-DIP universal screening study, when testing on the basis of the presence of one or more of the following risk factors; age ≥40 years, BMI ≥30 kg/m2, family history of diabetes, previous unexplained perinatal death, long-term steroid use, polyhydramnios or macrosomia in the current pregnancy, history of giving birth to a baby with a birth weight of ≥4.5 kg, polycystic ovary syndrome, current glycosuria, or ethnicity associated with a high risk of type 2 diabetes. As these women were tested outside of the ATLANTIC-DIP study, we do not have complete data on the total number of women screened, or diagnosed with GDM, outside of the universal screening portion of the study. A total of 675 women of white European origin were identified that met IADPSG criteria for GDM during the period 2007 – 2010. Of these, 262 women either declined or could not be contacted. Of those agreeing to participate, 72 (21%) did not attend, while 3 did not complete the test. 270 women (45% of eligible population) attended and completed retesting. A control group of 2254 women known to have NGT in pregnancy during this time period were invited for retesting, using a process of random selection. Of these, 500 agreed to participate - 112 (22%) did not attend, while 388 underwent and completed retesting. Comparison of the two groups' characteristics at testing for this study is shown in table 3.1.

Variable	GDM (n=270)	NGT (n=388)	95% CI for difference	p value for difference
Characteristics at retesting				
Age- years	36.6 (5.0)	37.6 (5.1)	0.13, 1.70	0.022
Years since delivery	2.6 (1.0)	3.3 (0.7)	0.6, 0.9	<0.001
BMI – kg/m^2	29.7 (6.9)	26.1 (4.9)	2.6, 4.5	<0.001
Waist circumference- cm	93.2 (16.3)	84.5 (12.5)	6.4, 11.1	<0.001
Fasting glucose - mmol/L	5.07 (0.95)	4.65 (0.41)	0.30, 0.54	<0.001
2-hr glucose - mmol/L	5.62 (1.75)	4.85 (1.02)	0.53, 1.02	<0.001
HbA1c IFCC - mmol/mol	36.2 (4.8)	33.6 (2.7)	1.9, 3.2	<0.001
HbA1c DCCT- %	5.56 (0.45)	5.32 (0.26)	0.18, 0.30	<0.001
Total cholesterol – mmol/L	5.0 (1.0)	4.7 (0.8)	0.2, 0.5	<0.001
Triglycerides- mmol/L	1.3 (0.9)	0.9 (0.5)	0.3, 0.5	<0.001
HDL-cholesterol- mmol/L	1.5 (0.4)	1.6 (0.4)	0.1, 0.2	<0.001
LDL-cholesterol – mmol/L	2.9 (0.8)	2.7 (1.0)	0.1, 0.4	<0.001
SBP- mmHg	124.7 (15.4)	116.1 (13.4)	6.4, 11.0	<0.001
DBP- mmHg	73.6 (10.4)	68.8 (9.2)	3.2, 6.3	\ <0.001

Variable	GDM (n=270)	NGT (n=388)	95% CI for difference	p value for difference
Proportion with family history of diabetes	65.2%	50.0%	7.5, 22.5	<0.001
Proportion with abnormal glucose tolerance at first post-partum visit	15.9%	0.9%	10.7, 19.6	<0.001
Characteristics at the time of the index pregnancy				
Age in years at time of delivery in index pregnancy	34.0 (5.0)	34.2 (5.1)	-1.0. 0.6	0.626
BMI in kg/m^2 at time of pregnancy OGTT**	31.3 (6.6)	27.1 (4.3)	3.2, 5.1	<0.001
SBP in mmHg at time of pregnancy OGTT***	122.8 (12.3)	119.1 (12.2)	1.7, 5.8	<0.001
DBP in mmHg at time of pregnancy OGTT***	73.9 (8.7)	71.0 (8.8)	1.5, 4.4	<0.001
Gestational week OGTT performed	27.3 (4.2)	26.4 (2.6)	0.4, 1.5	0.001
Pregnancy fasting glucose-mmol/L	5.16 (0.89)	4.31 (0.32)	0.74, 0.96	<0.001
1 hour glucose – mmol/L	10.37 (1.73)	6.75 (1.61)	3.4, 3.9	<0.001
2 hour glucose – mmol/L	8.13 (2.06)	5.42 (1.29)	2.4, 3.0	<0.001
Proportion using insulin in index pregnancy	35.6%	2.1%	27.7, 39.5	<0.001
Offspring birthweight in kg	3.47 (0.69)	3.56 (0.62)	-0.20, 0.01	0.076
Preeclampsia or pregnancy-induced hypertension in index pregnancy	24.9%	11.0%	8.1, 20.1	<0.001
Polyhydramnios	7.8%	2.8%	1.5, 9.0	0.005
Congenital malformation present	1.5%	4.1%	-0.1, 5.3	0.040

Variable	GDM (n=270)	NGT (n=388)	95% CI for difference	p value for difference
Shoulder dystocia	0.7%	3.1%	0.0, 4.7	0.053
Neonatal death	0.4%	1.8%	-0.5, 3.0	0.095
Neonatal intensive care unit admission required	22.2%	14.9%	1.3, 13.5	0.011
Caesarean section	47.8%	26.8%	13.5, 28.2	<0.001
Emergency Caesarean section	19.3%	11.3%	2.4, 13.8	0.005
Breastfeeding status relating to index pregnancy				
Any breastfeeding	59%	55.2%	-3.8, 11.5	0.184
Continued post hospital discharge	45.6%	53.4%	0.0, 15.4	0.029
Continued 1 month or more	41.5%	44.6%	-4.5, 10.7	0.239
Continued 3 months or more	31.9%	35.8%	-3.4, 11.2	0.165

Table 3.1. Characteristics of participants meeting criteria for International Association of Diabetes and Pregnancy Study Groups (IADPSG)-defined gestational diabetes (GDM) and normal glucose tolerance (NGT).
Figures represent means (standard deviation) unless otherwise stated.

** incomplete data set- 10% of GDM and 16% of NGT participants did not have values recorded
*** incomplete data set- 15% of GDM and 16% of NGT participants did not have values recorded

The mean duration of follow-up was shorter in women with previous IADPSG-defined GDM [2.6 (SD 1.0) years versus 3.3 (SD 0.7) years in women with NGT ($p < 0.001$)]. A boxplot detailing the distribution of time

from index pregnancy to retesting is shown in Fig 3.2. Women with IADPSG-defined GDM were also younger than those with NGT in pregnancy (36.6 [SD 5.0] vs. 37.6 [5.1] years, p=0.022). Women with previous GDM had a higher BMI, higher glucose and HbA1c levels, Higher LDL-cholesterol and triglycerides, with lower HDL-cholesterol, and higher blood pressure readings. They also had higher mean glucose values at each point on the pregnancy OGTT, and were more likely to have a family history of diabetes. Insulin use during pregnancy, as expected, was higher among those with IADPSG-defined GDM. However, the NGT group contained some women who received insulin therapy during pregnancy, as this group contains a number of women meeting WHO 2-hr criteria for diagnosis of GDM, which as mentioned in the methods chapter, were in clinical use at the time of the study. In addition, women with isolated elevation of the one-hour glucose value (which were not included in the 1999 WHO diagnostic criteria) were instructed in self-monitoring of blood glucose and given dietary advice. If fasting (<5.3 mmol/L) and 1-hour postprandial (<7.8 mmol/L) glucose targets were not met with dietary therapy, insulin therapy was commenced.

3.2.2 Prevalence of abnormal glucose tolerance

Of the 270 women with a history of GDM by IADPSG criteria, 10.4% (n=28) were found to have a new diagnosis of AGT (Table 3.2). In addition to the 15.6% (n= 42) of women already known to have AGT at initial postpartum testing, this yielded an overall prevalence of AGT in this cohort of 25.9% in women with prior IADPSG-defined GDM, compared to 3.6% (n=14) of those with NGT (p = 0.001). Of the 270 women with a history of GDM, 12.2% had IFG, 5.9% had IGT, 5.6% had combined IFG/IGT, while 2.2% had DM. Of the 388 women with previous NGT, 1.8% had IFG, 1.5% had IGT, 0.3% had combined IFG/IGT, and none had DM (Table 3.2).

Within the GDM group, as compared with women with normal glucose tolerance post-GDM (table 3.3) women diagnosed with AGT since the

index pregnancy were the same age (34.1 years, SD 5.0, p=0.756), had a higher BMI (32.17 kg/m^2, SD 6.52 vs. 28.78 kg/m^2, SD 6.82, p<0.001), were diagnosed with GDM earlier in the index pregnancy (25.6 weeks, SD 5.5 vs. 28.0 weeks; SD 3.4, p<0.001), and had higher mean fasting (5.73 mmol/L, SD 1.1 vs. 4.96 mmol/L, SD 0.70, p<0.001), one-hour (10.83 mmol/L, SD 1.87 vs. 10.21 mmol/L, SD 1.65, p=0.017, and 2-hour (8.97 mmol/L, SD 2.50 vs. 7.83 mmol/L , SD 1.80, p<0.001) glucose levels on the 75g OGTT during pregnancy. A greater proportion of women with previous GDM progressing to AGT also had a family history of diabetes as compared with those who did not (75.7% vs. 61.5%, p=0.032).

With regard to the cohort of women with GDM who had abnormal glucose tolerance at early post-partum testing (n=42), 38% (n=16) had values consistent with normoglycaemia at retesting for this study (FPG < 5.6 mmol/L, 2hr plasma glucose < 7.8 mmol/L, and HbA1c < 5.7% /39 mmol/mol). 14% (n=6) had progressed to a higher category of abnormal glucose tolerance, while 48% (n=20) showed no change in category of abnormal glucose tolerance between the first post-partum test and retesting for this study. Of note, HbA1c values were included in the definition of normoglycaemia (HbA1c <5.7%, as per ADA criteria)(151) in this analysis, as 48% (n=20) of women with a previous diagnosis of abnormal glucose tolerance did not have a 2-hr post glucose load measured, as specified in the methods chapter. Women who had reverted to normoglycaemia at retesting had a significantly lower fasting glucose on the pregnancy OGTT, were significantly less likely to have used insulin during pregnancy, and had a significantly lower BMI at retesting, as compared with women who did not revert to normoglycaemia. Full details can be seen in table 3.4.

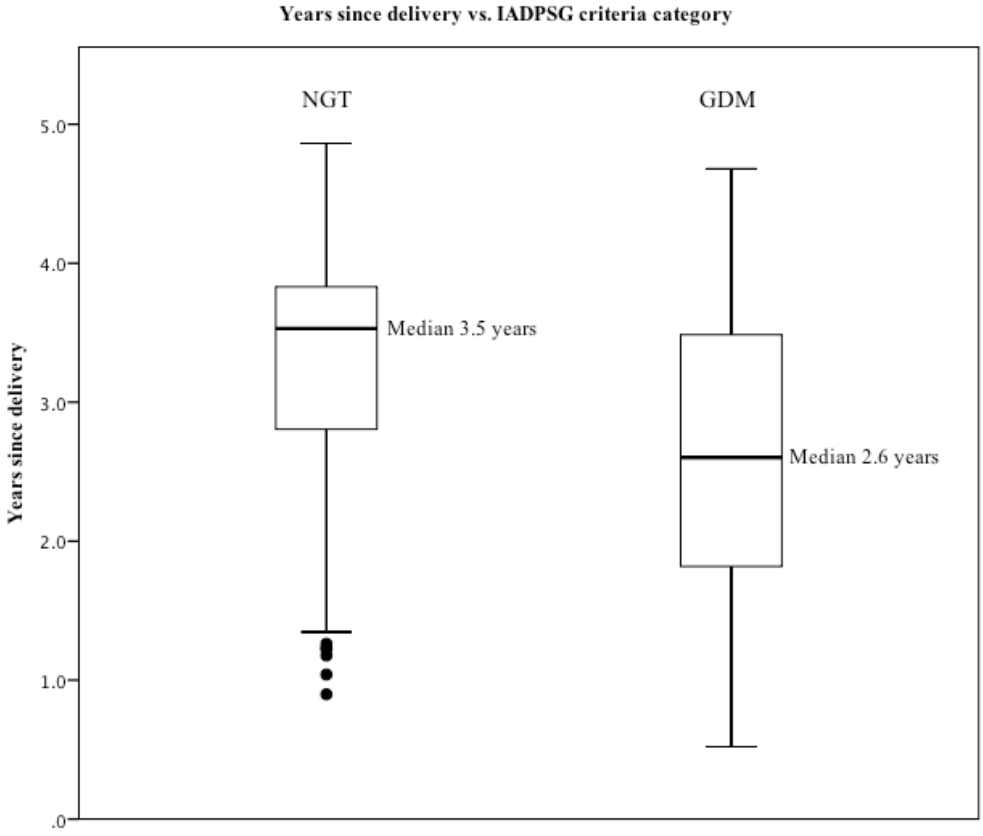

Fig 3.2. Boxplot of distribution of time elapsed since index pregnancy by GDM status. Outliers are indicated by closed circles.

GDM- gestational diabetes
NGT normal glucose tolerance
IADPSG- International Association of Diabetes and Pregnancy Study Groups

	GDM (n=270)	NGT (n=388)	95% CI for difference	p value for difference
Number of participants abnormal at early post-partum testing				
IFG	18	0		
IGT	8	2		
IFG/IGT	12	1		
DM	4	0		
Total early post -partum	42 (15.6%)	3 (0.8%)	10.7, 19.6	0.001
Number of participants with newly diagnosed abnormal glucose tolerance at this study				
IFG	15	7		
IGT	8	4		
IFG/IGT	3	0		
DM	2	0		
Total for this study	28 (10.4%)	11 (2.8%)	3.8, 11.9	<0.001
Total number	**70 (25.9%)†**	**14 (3.6%)**	**16.9, 28.0**	**0.001**

Table 3.2. Proportion of women with abnormal glucose tolerance at 12-week post-partum testing and at retesting for the current study.

GDM- Gestational diabetes, NGT- Normal glucose tolerance (both International Association of Diabetes and Pregnancy Study Groups criteria)
IFG- Impaired Fasting Glucose, IGT- Impaired Glucose Tolerance, DM- Diabetes Mellitus (All by American Diabetes Association criteria)

† added and calculated percentages differ slightly due to rounding error

	Abnormal glucose tolerance post-GDM (n=70)	**Normal glucose tolerance post-GDM** (n=200)	**95% CI for difference between groups**	**p value for difference between groups**
Index pregnancy variables				
Mean age at delivery - years (SD)	34.1 (5.0)	34.1 (5.0)	-1.1, 1.6	0.756
***Mean BMI at time of pregnancy OGTT - kg/m^2 (SD)**	32.84 (5.80)	30.73 (6.84)	0.23, 3.99	0.028
Median parity (range)	1 (0,6)	1 (0,5)	N/A	0.601
Mean gestational week tested (SD)	25.6 (5.5)	28.0 (3.4)	1.0, 3.8	<0.001
Mean fasting glucose on pregnancy OGTT - mmol/L (SD)	5.73 (1.1)	4.96 (0.70)	0.50, 1.06	<0.001
Mean 1- hour glucose on pregnancy OGTT - mmol/L (SD)	10.83 (1.87)	10.21 (1.65)	0.59, 1.69	0.017
Mean 2-hour glucose on pregnancy OGT - mmol/L (SD)	8.97 (2.50)	7.83 (1.80)	0.50, 1.79	0.001
Proportion with family history of diabetes	75.7%	61.5%	1.3, 25.2	0.032
Insulin use during pregnancy - proportion	48.6%	31%	4.4, 30.5	0.008
Mean infant birthweight - kg (SD)	3.46 (0.83)	3.47 (0.64)	-0.18, 0.20	0.932
Breastfeeding after birth- proportion	44.3%	59%	11.8, 27.6	0.037
Preeclampsia/pregnancy-induced hypertension- proportion	28.6%	23.6%	-17.7, 6.1	0.410
Polyhydramnios- proportion	2.9%	9.5%	-1.1, 11.9	0.116
Congenital malformations present - proportion	4.3%	0.5%	0.2, 11.3	0.055

	Abnormal glucose tolerance post-GDM (n=70)	**Normal glucose tolerance post-GDM** (n=200)	**95% CI for difference between groups**	**p value for difference between groups**
Shoulder dystocia present - proportion	1.4%	0.5%	-7.2, 1.6	0.452
Neonatal death- proportion	0%	0.5%	-4.7, 2.8	1.000
Neonatal ICU admission - proportion	28.6%	20%	-11.1, 1.8	0.138
Caesarean section – total proportion	62.9%	42.5%	6.8, 32.7	0.003
Emergency Caesarean section - proportion	21.4%	18.5%	-14.9, 7.0	0.600
Variables at follow-up (up to 5 years postpartum)				
Mean age at follow-up - years (SD)	36.5 (4.9)	36.7 (5.0)	-1.2, 1.5	0.806
Mean years since delivery (SD)	2.6 (1.0)	2.6 (1.0)	-0.3, 0.2	0.763
Mean BMI - kg/m^2 (SD)	32.17 (6.52)	28.78 (6.82)	1.55, 5.24	<0.001
Ever smoked- proportion	47.1%	53.8%	-7.1, 19.5	0.340
Alcohol use –proportion	58.6%	53.6%	-19.4, 7.0	0.474
Continued breastfeeding at home- proportion	35.7%	49.0%	-0.3, 25.6	0.055
Continued breastfeeding for I month or more – proportion	32.9%	44.5%	-1.8, 2.4	0.089
Continued breastfeeding for 3 months or more- proportion	30.0%	32.5%	-10.6, 14.1	0.699
Mean fasting glucose - mmol/L (SD)	5.83 (1.52)	4.81 (0.40)	0.65, 1.38	<0.001

	Abnormal glucose tolerance post-GDM (n=70)	**Normal glucose tolerance post-GDM** (n=200)	**95% CI for difference between groups**	**p value for difference between groups**
Mean 2-hr glucose mmol/L (SD)	7.43 (2.68)	5.18 (1.04)	1.44, 3.04	<0.001
Mean HbA1c - mmol/mol	39.0 (7.3)	35.2 (3.0)	2.0, 5.6	<0.001
Mean HbA1c -%	5.83 (0.68)	5.47 (0.28)	0.19, 0.53	<0.001
Mean SBP - mmHg (SD)	129.3 (16.8)	123.1 (14.6)	2.0, 10.3	0.004
Mean DBP - mmHg (SD)	76.5 (11.0)	72.6 (10.0)	1.1, 6.7	0.007

Table 3.3. Characteristics of women with IADPSG-defined GDM who were diagnosed with abnormal glucose tolerance after delivery (up to 5 years post-partum) as compared with those who still have NGT at follow-up.

IADPSG-International Association of Diabetes and Pregnancy Study Groups ;
GDM- IADPSG-defined GDM; NGT- normal glucose tolerance by IADPSG criteria
SD- standard deviation, CI- confidence interval; OGTT- oral glucose tolerance test; BMI- Body Mass Index; SBP- systolic blood pressure; DBP- diastolic blood pressure

Variable	Persistent abnormal glucose tolerance (n=26)	Reverted to normal glucose tolerance (n=16)	95% CI for difference	p value for difference
Characteristics at the time of the index pregnancy				
Age in years at time of delivery in index pregnancy	34.4 (5.5)	33.6 (3.6)	-2.4, 3.9	0.639
BMI in kg/m^2 at time of pregnancy OGTT	34.0 (6.9)	32.2 (4.3)	1.74, 1.92	0.370
SBP in mmHg at time of pregnancy OGTT	122.4 (12.6)	122.5 (10.0)	-7.9, 7.6	0.964
DBP in mmHg at time of pregnancy OGTT	75.9 (9.4)	75 (8.0)	0.753	-5.0, 6.8
Gestational week OGTT performed	24.7 (5.2)	25.0 (4.9)	-3.6, 2.9	0.831
Pregnancy fasting glucose-mmol/L	5.94 (0.87)	5.76 (1.66)	-0.76, 1.12	0.696
1 hour glucose – mmol/L	11.33 (1.59)	10.96 (1.95)	-0.79, 1.54	0.520
2 hour glucose – mmol/L	9.29 (2.80)	9.44 (1.88)	-1.76, 1.45	0.846
Proportion using insulin in index pregnancy	37.5%	69.2%	1.1, 55.5	0.044
Offspring birthweight in kg	3.21 (0.73)	3.49 (0.78)	-0.78, 0.19	0.234
Preeclampsia or pregnancy-induced hypertension in index pregnancy (%)	34.6%	18.8%	-12.8, 38.6	0.269
Polyhydramnios (%)	0%	0%	N/A	N/A
Congenital malformation present (%)	7.7%	0%	-12.5, 24.1	0.256
Shoulder dystocia (%)	3.8%	0%	-15.8, 18.9	0.427

Variable	Persistent abnormal glucose tolerance (n=26)	Reverted to normal glucose tolerance (n=16)	95% CI for difference	p value for difference
Neonatal death (%)	0%	0%	N/A	N/A
Neonatal intensive care unit admission required (%)	26.9%	18.8%	-19.5, 30.9	0.546
Caesarean section (%)	61.5%	81.3%	-42.3, 9.4	0.180
Emergency Caesarean section (%)	15.4%	37.5%	-47.7, 4.2	0.102
Breastfeeding status relating to index pregnancy				
Any breastfeeding (%)	38.5%	18.8%	-9.4, 42.3	0.180
Continued post hospital discharge (%)	23.1%	12.5%	-15.9, 31.6	0.397
Continued 1 month or more (%)	19.2%	12.5%	-19.1, 27.4	0.570
Continued 3 months or more (%)	15.4%	12.5%	-22.4, 23.1	0.795
Characteristics at retesting for this study				
Age- years	36.7 (5.0)	36.3 (3.7)	-2.40, 3.86	0.639
Years since delivery	2.3 (1.0)	2.7 (1.1)	-1.01, 0.35	0.344
BMI – kg/m^2	33.7 (7.1)	28.9 (3.9)	1.33, 8.19	0.008
Waist circumference- cm	101.7 (16.9)	94.7 (9.6)	-2.3, 16.5	0.135
Fasting glucose - mmol/L	6.39 (2.18)	5.17 (0.56)	0.09, 2.35	0.034
2-hr glucose - mmol/L	8.46 (3.54)	5.66 (0.51)	0.53, 5.06	0.020

Variable	Persistent abnormal glucose tolerance (n=26)	Reverted to normal glucose tolerance (n=16)	95% CI for difference	p value for difference
HbA1c IFCC - mmol/mol	42.7 (9.7)	34.8 (2.3)	2.85, 12.89	0.003
HbA1c DCCT- %	6.17 (0.92)	5.43 (0.21)	0.27, 1.21	0.003
Total cholesterol – mmol/L	5.15 (1.06)	4.79 (1.0)	-0.30, 1.0	0.281
Median triglycerides- mmol/L (IQR)	1.30 (0.98, 1.8)	1.10 (0.90, 1.60)	-0.23, 0.63	0.323
HDL-cholesterol- mmol/L	1.37 (0.57)	1.35 (0.34)	-0.29, 0.34	0.884
LDL-cholesterol – mmol/L	2.85 (0.60)	2.86 (0.93)	-0.50, 0.48	0.959
Median HOMA2-IR (IQR)	2.7 (1.65, 4.55)	1.6 (1.00, 1.28)	0.05, 2.15	0.028
SBP- mmHg	132.8 (16.5)	124.9 (15.66)	-0.26, 18.2	0.137
DBP- mmHg	79.5 (9.40)	70.9 (10.9)	2.2, 14.9	0.10
Proportion with family history of diabetes (%)	76.9%	75.0%	-22.2, 29.2	0.887

Table 3.4 Characteristics of women with IADPSG-defined GDM diagnosed with abnormal glucose tolerance post-partum. This table shows a comparison of those who demonstrated persistent hyperglycaemia at follow-up for this study versus those who did had reverted to normoglycaemia.

IADPSG-International Association of Diabetes and Pregnancy Study Groups ;
GDM- IADPSG-defined GDM; NGT- normal glucose tolerance by IADPSG criteria
SD- standard deviation, CI- confidence interval; OGTT- oral glucose tolerance test; BMI- Body Mass Index; SBP- systolic blood pressure; DBP- diastolic blood pressure

Comparison with women who did not attend

As outlined above, one of the original study centres was omitted from follow-up due to economic and practical considerations. Women with IADPSG-defined GDM from the omitted centre (n=79), as compared with all other centres, were of similar age (33.1 [SD 4.9] vs. 33.6 [SD 5.4] years, p=0.441), had a similar BMI (30.8 [SD 6.3] vs. 31.2 [SD 6.6] kg/m^{2,}, p=0.629), but lower fasting glucose (4.93 [SD 0.69] vs. 5.17 [SD 0.84] mmol/L, p=0.015), and similar 1-hour (10.30 [SD 1.87] vs. 9.84 [SD1.98] mmol/L, p=0.067) and higher 2-hour glucose (8.91 [SD 1.89] vs. 7.54 [SD 2.09] mmol/L, p <0.01) values on the pregnancy OGTT.

As compared with those women with IADPSG-defined GDM participating in this study, those who participated in the original ATLANTIC-DIP study but did not attend for this study (n=318) were of similar age (33.2 [SD 5.7] vs. 34.0 [SD 5.0] years, p=0.052), had a similar BMI during pregnancy (31.37 [SD 6.64] vs. 31.16 [SD 6.57] kg/m^{2,} p=0.729), and a similar fasting glucose (5.16 [SD 0.89] vs. 5.19 [SD 0.81] mmol/L, p=0.664). They did, however, have lower 1-hour (9.40 [SD 2.06] vs. 10.37 [SD 1.73] mmol/L, p<0.001), and 2-hour (7.04 [SD 1.99] vs. 8.13 [SD 2.06] mmol/L, p<0.001) glucose values on the pregnancy 75g OGTT. Non-attenders were less likely to have used insulin during pregnancy, and were less likely to have a family history of diabetes. Full details are shown in table 3.5. The higher proportion of these risk factors in those who attended raises the possibility that the true prevalence of AGT in the population may be overestimated when calculations are based on this cohort. However, the strongest and most consistent risk factor seen in the literature for the prediction of progression to diabetes post-GDM, that of the level of fasting glucose on the pregnancy OGTT, does not differ between the groups. Also, as we will see , the risk of bias towards a higher prevalence of abnormal glucose tolerance in attenders vs. non-attenders is also present in the NGT group.

	Women with GDM who attended for follow-up (n=270)	**Women with GDM who did not attend for follow-up** (n=318)	**95% CI for difference between groups**	**p value for difference between groups**
Age at delivery – years (SD)	34.0 (5.0)	33.2 (5.7)	-0.01, 1.71	0.052
Mean fasting glucose on pregnancy OGTT - mmol/L (SD)	5.16 (0.89)	5.19 (0.81)	-0.17, 0.11	0.664
Mean 1- hour glucose on pregnancy OGTT - mmol/L (SD)	10.37 (1.73)	9.40 (2.06)	0.66, 1.3	<0.001
Mean 2-hour glucose on pregnancy OGTT - mmol/L (SD)	8.13 (2.06)	7.04 (1.99)	0.76, 1.41	<0.001
Mean gestational week tested (SD)	27.3 (4.2)	27.4 (3.7)	-0.7, 0.5	0.757
Infant birthweight - kg (SD)	3.47 (0.69)	3.55 (0.60)	-0.19, 0.02	0.112
***Mean BMI at time of pregnancy OGTT - kg/m^2 (SD)**	31.37 (6.64)	31.16 (6.57)	-0.97, 1.39	0.729
Proportion with family history of diabetes	65.2%	44.3%	12.8, 28.5	<0.001
Polyhydramnios	7.4%	2.6%	1.3, 8.8	0.007
Insulin use during pregnancy	35.6%	14.5%	14.1, 27.9	<0.001
Shoulder dystocia	0.7%	1.6%	-1.2, 3.1	0.456
Neonatal ICU admission	22.7%	17.6%	1.8, 11.2	0.263
Caesarean section (both elective and emergency)	47.8%	40.2%	-1.6, 0.5	0.067

Table 3.5 Pregnancy characteristics of women with IADPSG-defined GDM who attended for follow-up in this study, compared with those who did not attend for follow-up for this study, but were screened during the ATLANTIC-DIP universal screening programme.

The characteristics of women with NGT who attended for retesting for this study, as compared with those who did not, is demonstrated in Table 3.6. Women with NGT who attended for follow-up were older, had a higher mean 2-hour glucose on the pregnancy OGTT, were more likely to have a family history of diabetes, and were more likely to have used insulin during pregnancy (note some women with WHO-defined GDM, are defined as NGT using the IADPSG criteria). Therefore, as the NGT population attending for retesting have higher-risk characteristics for the development if type 2 diabetes, the true prevalence of AGT in the NGT group may be overestimated in our cohort. This higher-risk status may also be reflected in the relatively high number of neonatal deaths (7 in the NGT group attending for retesting for this study, of whom 6 had congenital malformations). Of note , women with NGT who suffered a neonatal death (n=7), as compared with those who did not (n=381), were older at delivery (38.2 vs. 34.2 years, p=0.40), but showed no difference in BMI, blood pressure values, or glucose levels on the OGTT.

Comparison among subgroups

When data on women attending for this study were analysed according to WHO 1999 criteria, 214 women met criteria for GDM diagnosis, while 444 met NGT criteria. The prevalence of abnormal glucose tolerance was 29.4% among women with WHO-defined GDM and 4.7% among women with WHO-defined NGT. Neither of these proportions differed significantly from those seen when IADPSG criteria were used (p=0.390 for women with GDM, p=0.421 for women with NGT). One hundred and seventy-five women met both IADPSG and WHO 1999 criteria, 95 met IADPSG criteria only, 39 met WHO 1999 criteria only, and 349 met neither IADPSG nor WHO criteria. Women meeting both IADPSG and WHO 1999 criteria had a significantly greater prevalence of abnormal glucose tolerance at follow-up than those meeting IADPSG criteria alone (prevalence 32.6% vs. 13.7%, $p<0.001$), or women meeting WHO 1999 criteria alone (prevalence 32.6% vs. 15.4%, p=0.033).

	Women with NGT who attended for follow-up (n=388)	Women with NGT who did not attend for follow-up (n=3563)	95% CI for difference between groups	p value for difference between groups
Age at delivery – years (SD)	34.2 (5.1)	31.7 (5.3)	1.9, 3.1	<0.001
Mean fasting glucose on pregnancy OGTT - mmol/L (SD)	4.31 (0.32)	4.30 (0.31)	-0.04, 0.02	0.575
Mean 1- hour glucose on pregnancy OGTT - mmol/L (SD)	6.75 (1.61)	6.61 (1.42)	-0.31, 0.03	0.102
Mean 2-hour glucose on pregnancy OGTT - mmol/L (SD)	5.42 (1.29)	5.12 (1.09)	0.17. 0.43	<0.001
Mean gestational week tested (SD)	26.4 (2.6)	26.5 (2.3)	-0.2, 0.3	0.675
Infant birthweight - kg (SD)	3.56 (0.62)	3.55 (0.55)	-0.19, 0.02	0.112
***Mean BMI at time of pregnancy OGTT - kg/m^2 (SD)**	27.03 (4.30)	26.37 (4.68)	0.13, 1.19	0.015
Proportion with family history of diabetes	50%	28%	16.9, 27.2	<0.001
Polyhydramnios	1.3%	0.8%	-0.23, 0.3	0.255
Insulin use during pregnancy	2.1%	0.1%	0.9, 3.9	<0.001
Shoulder dystocia	1.6%	1.3%	-2.1, 0.7	0.702
Neonatal ICU admission	22.7%	17.6%	4.0, 11.1	0.263
Caesarean section (both elective and emergency)	26.7%	26.0%	-5.5, 3.7	0.771

Table 3.6. Pregnancy characteristics of women with IADPSG-defined NGT who attended for follow-up in this study, compared with those who did not attend for follow-up for this study, but were screened during the ATLANTIC-DIP universal screening programme.

There was no significant difference in the prevalence of abnormal glucose tolerance at follow-up when women meeting IADPSG criteria only were compared with those meeting WHO 1999 criteria only (p=0.798). However, it should be noted that not all women meeting WHO criteria were recalled for screening- those with a 2-hr glucose of 7.8-8.4 mmol/L would have been subject to random selection for invitation for retesting. Overall 44% of women meeting WHO criteria from the original ATLANTIC-DIP universal screening cohort were screened for this study.

Also of note, women identified as having IADPSG-defined GDM during the universal screening period, when compared with those identified using risk-factor based screening, were slightly younger at delivery (33.6 [SD 5.0] vs. 35.2 [SD 4.8] years, p=0.024), and had a lower (10.12 [SD 1.74] vs. 11.2 [SD 1.42] mmol/L, p<0.001) one-hour, but similar fasting (5.15 [SD 0.85] vs. 5.18 [SD 1.02] mmol/L, p=0.790) and 2-hour (8.04 [SD 2.02] vs. 8.39 [SD 2.19] mmol/L, p=0.235) glucose values. Other pregnancy characteristics were similar, aside from an increased prevalence of hypertension or pre-eclampsia during pregnancy in those women identified during risk factor-based screening (35.4% vs. 21.6%, p=0.025). Further details are available in table 3.7. Despite this, no difference was seen in the proportion of women with abnormal glucose tolerance post-delivery (24.6% for risk factor-based screening vs. 26.3%, for universal screening, p=0.782).

	Universal screening (n=205)	Risk-factor based screening (n=65)	95% CI for difference between groups	p value for difference between groups
Index pregnancy variables				
Mean age at delivery - years (SD)	33.6 (5.0)	35.2 (4.8)	-3.0, -0.2	0.024
***Mean BMI at time of pregnancy OGTT - kg/m^2 (SD)**	31.1 (6.8)	32.1 (6.2)	-3.0, 1.0	0.331
Median parity (range)	1 (0.6)	1 (0,3)	N/A	0.833
Mean gestational week tested (SD)	27.1 (4.3)	28.1 (3.9)	-2.2, 0.1	0.077
Mean fasting glucose on pregnancy OGTT - mmol/L (SD)	5.15 (0.85)	5.18 (1.02)	-0.28, 0.26	0.790
Mean 1- hour glucose on pregnancy OGTT - mmol/L (SD)	10.12 (1.74)	11.20 (1.42)	-1.5, -0.5	<0.001
Mean 2-hour glucose on pregnancy OGT - mmol/L (SD)	8.04 (2.02)	8.39 (2.19)	-0.93, 0.23	0.235
Proportion with family history of diabetes	64.4	67.7	-15.5, 10.3	0.626
Insulin use during pregnancy	32.7	44.6	-25.4, 1.3	0.080
Infant birthweight - kg (SD)	3.51 (0.67)	3.34 (0.72)	-0.17, 0.37	0.075
Breastfeeding after birth- proportion	2.7	63.1	-23.1, 3.5	0.142
Preeclampsia/pregnancy-induced hypertension	21.6%	35.4%	1.6, 27.0	0.025
Polyhydramnios	7.8%	7.7%	-9.4, 6.4	0.976
Congenital malformations	2.0%	0.0%	-3.8 4.9	0.257
Shoulder dystocia	1%	0%	-4.7, 3.5	0.424

	Universal screening (n=205)	Risk-factor based screening (n=65)	95% CI for difference between groups	p value for difference between groups
Neonatal death	0.5%	0%	-5.1, 2.7	0.573
Neonatal ICU admission	20.5%	27.7%	-20.1, 4.0	0.223
Emergency Caesarean section	18.5%	21.5%	-15.4, 7.1	0.593
Variables since the index pregnancy				
Mean age at follow-up - years (SD)	36.7 (5.1)	36.6 (1.8)	-1.3, 1.5	0.893
Mean years since delivery (SD)	3.0 (0.8)	1.3 (0.6)	1.5, 1.9	<0.01
Mean BMI - kg/m^2 (SD)	29.75 (7.26)	29.37 (5.63)	-1.33, 2.10	0.660
Ever smoked- proportion	51.5%	53.8%	-15.8, 11.4	0.738
Alcohol use –proportion	52.5%	62.5%	-22.9, 4.0	0.162
Continued breastfeeding at home- proportion	44.9%	47.7%	-16.5, 10.7	0.691
Continued breastfeeding for I month or more – proportion	40.5%	44.6%	-17.8, 9.2	0.556
Continued breastfeeding for 3 months or more- proportion	31.7%	32.3%	-14.1, 11.5	0.928
Mean fasting glucose - mmol/L (SD)	5.10 (1.04)	5.00 (0.63)	-0.17, 0.37	0.466
Mean 2-hr glucose mmol/L (SD)	5.55 (1.81)	5.85 (1.53)	-0.11, 0.31	0.257
Mean HbA1c - mmol/mol	36.2 (5.0)	36.3 (3.9)	-1.4, 1.3	0.946
Mean HbA1c -%	5.56 (0.47)	5.57 (0.37)	-0.13, 0.12	0.946

	Universal screening (n=205)	Risk-factor based screening (n=65)	95% CI for difference between groups	p value for difference between groups
Mean SBP - mmHg (SD)	124.2 (15.1)	126.5 (16.5)	-6.6, 2.0	0.299
Mean DBP - mmHg (SD)	73.2 (10.3)	74.9 (10.6)	-4.6, 1.2	0.259

Table 3.7. Characteristics of women with IADPSG-defined GDM identified during the universal screening programme as compared with those identified during risk-factor based screening.

3.2.3 Prediction of progression to abnormal glucose tolerance

In order to test the ability of routinely available clinical variables to predict which women will demonstrate AGT, both at early post-partum testing and follow-up for this study, we first constructed a decision tree model. This model included variables from both the index pregnancy, and also at further follow-up. The model was restricted to those participants with a full dataset on all modelled variables (n=595). The decision tree process used classification and regression tree analysis (CART)(149) and (Fig 3.3) identified the following predictive variables (in order of importance); fasting glucose on pregnancy OGTT; birthweight; age at follow-up; one-hr glucose on pregnancy OGTT; BMI at follow-up; two-hour glucose on pregnancy OGTT; number of years since delivery; gestational week tested for GDM; admission to the neonatal unit in the index pregnancy; emergency Caesarean section required; pregnancy-induced hypertension or pre-eclampsia; insulin use during pregnancy; number of subsequent pregnancies with GDM recurrence. The model derived using these showed high specificity of 91.3%, while sensitivity was 30.6%.

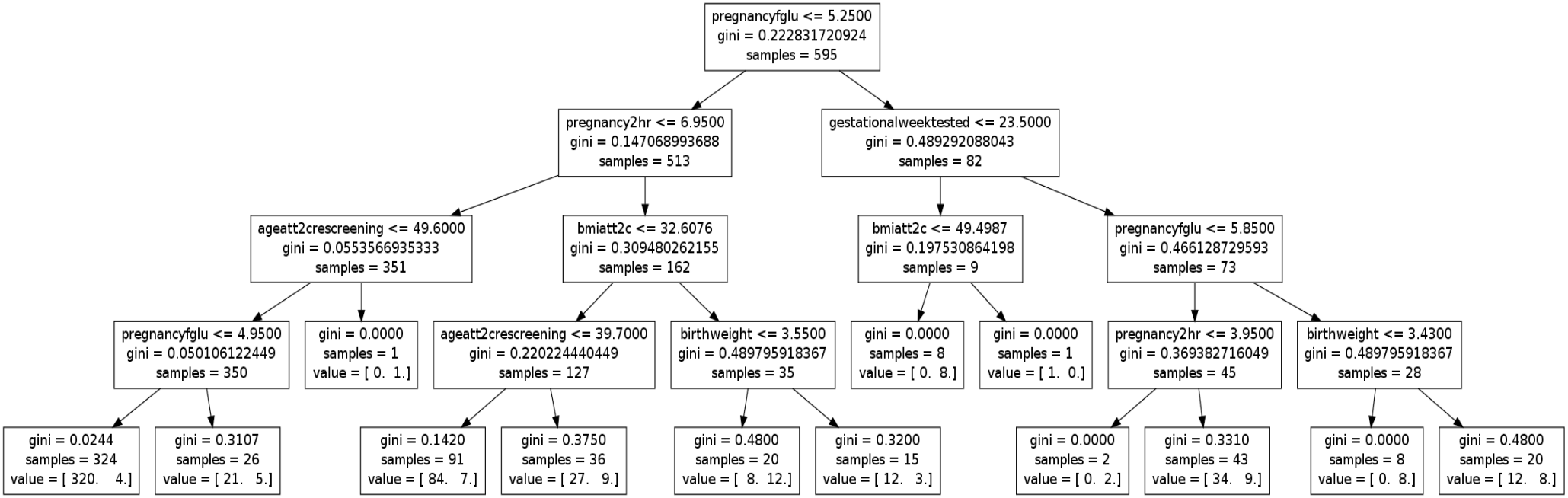

Fig 3.3 Decision tree for prediction of abnormal glucose tolerance in women with a previous history of gestational diabetes by IADPSG criteria. Index pregnancy variables were used to create a decision tree using a CART (Classification and Regression Tree) algorithm and the Gini splitting criterion. Pruning was also carried out to improve predictive ability and reduce the overall complexity of the model. The following features were identified (in order of importance); fasting glucose on pregnancy OGTT; birthweight; age at follow-up; one-hr glucose on pregnancy OGTT; BMI at follow-up; two-hour glucose on pregnancy OGTT; number of years since delivery; gestational week tested for GDM; admission to the neonatal unit in the index pregnancy; emergency Caesarean section required; pregnancy-induced hypertension or pre-eclampsia; insulin use during pregnancy; number of subsequent pregnancies with GDM recurrence.

Pregnancyfglu *–Fasting glucose value on pregnancy OGTT (mmol/L),* ***Pregnancy2hr****- 2hr glucose value on pregnancy OGTT (mmol/L),*
Ageatt2crescreening*- age at retesting for the current study (years),* ***BMIatt2c****- BMI at retesting for the current study(kg/m²),* ***gini****-Gini impurity index*

We also used logistic regression analyses to construct predictive models, again looking at total AGT at up to 5 years. We constructed 2 models, one using variables available at the time of delivery only (n=523), and one adding variables available at follow-up testing for this study (n=595). Variables that were significant on univariable analysis or yielded information gain of greater than 5% in the decision tree analysis were included in the multivariable model. The results of the logistic regression analysis are outlined in Table 3.8. Fasting glucose value on the pregnancy OGTT proved the strongest predictor on both models. Two-hr glucose on the pregnancy OGTT, earlier gestational week tested for GDM, and family history of diabetes also predicted AGT at follow-up in both models. BMI at follow-up also predicted AGT, while BMI during pregnancy did not. One-hour glucose on the pregnancy OGTT was a significant predictor in the model using index pregnancy variables only. Pseudo-r^2 values were 0.260 for the model with pregnancy variables only and 0.299 for the full model (including follow-up variables). With regard to overall predictive capability, these models displayed suboptimal performance, showing a high specificity of 97.6% and a sensitivity of 25.8% for prediction of AGT using index pregnancy variables only. Using the model that included postpartum variables, specificity remained high at 97.7%, while sensitivity was still low at 31.0%. Finally, a visual representation of the ability to discriminate women with AGT from those with NGT is shown in Fig 3.4, using multidimensional scaling (MDS), a technique that uses uses a non-linear mapping to transform measurements in a high-dimensional space (all continuous variables available in the study- see Table 3.1) down to 2 dimensions for simple visualization (152). This cohort demonstrates heterogeneous distribution and poor separation between women with AGT and those with NGT.

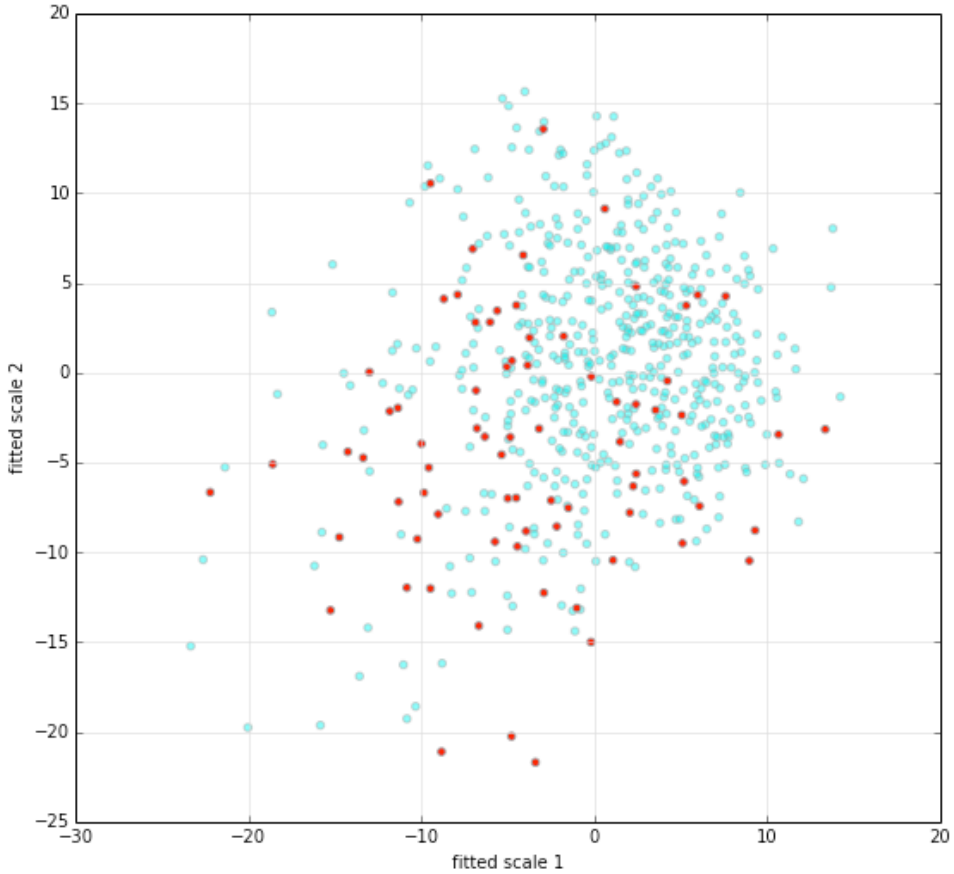

Fig 3.4. Scatterplot of women progressing to abnormal glucose tolerance up to 5 years post-partum (red) versus those with sustained normal glucose tolerance (blue) created using Multidimensional Scaling (MDS). MDS uses a non-linear mapping to transform measurements in a high-dimensional space (all continuous variables available in the study) down to 2 dimensions for simple visualisation. When we indicate the class of glucose tolerance with color, we see a heterogeneous distribution and poor separation of women with abnormal glucose tolerance (red) and normal glucose tolerance (blue). This analysis supports the findings of the classification models used in the investigation, which showed poor discriminative performance.

	Model using index pregnancy variables only		**Model including variables available at follow-up**	
	pseudo r^2=0.260		pseudo r^2=0.299	
	(n=523)		(n=595)	
Variable	**Odds ratio (95% CI)**	**p value**	**Odds ratio (95% CI)**	**p value**
Fasting glucose on pregnancy OGTT (per mmol/L)	2.79 (1.69, 4.59)	<0.01	2.33 (1.46, 3.71)	<0.01
1-hr glucose on pregnancy OGTT (per mmol/L)	1.20 (1.00, 1.42)	0.045	1.19 (0.99, 1.43)	0.059
2-hr glucose on pregnancy OGTT (per mmol/L)	1.21 (1.03, 1.43)	0.022	1.30 (1.1,1.5)	0.002
Week of gestation tested for GDM (per additional week)	0.92 (0.84, 1.00)	0.048	0.90 (0.83, 0.98)	0.018
BMI during pregnancy (per kg/m^2)	1.00 (0.95, 1.06)	0.914	N/A	N/A
Age at delivery (per year)	0.99 (0.93, 1.05)	0.781	N/A	N/A
Pre-term delivery (per day)	1.19 (0.63, 2.25)	0.595	1.01 (0.54, 1.90)	0.971
Pregnancy-induced hypertension/pre-eclampsia	0.76 (0.35, 1.65)	0.492	0.56 (0.26, 1.20)	0.138
Emergency Caesarean delivery	1.12 (0.53, 2.36)	0.768	1.30 (0.62, 2,71)	0.487
Birthweight (per kg)	0.93 (0.59, 1.45)	0.742	0.90 (0.58, 1.41)	0.660
Insulin use during pregnancy	0.85 (0.40, 1.81)	0.676	1.19 (0.56, 2.51)	0.648
Age at follow-up (per year)	N/A	N/A	0.98 (0.92, 1.04)	0.572
BMI at follow-up (per kg/m^2)	N/A	N/A	1.05 (1.01, 1.10)	0.020
Family history of diabetes	2.21 (1.13, 4.30)	0.019	2.25 (1.16,4.35)	0.017
Neonatal unit admission	1.16 (0.56, 2.42)	0.692	0.91 (0.43, 1.92)	0.798

	Model using index pregnancy variables only		Model including variables available at follow-up	
	pseudo r^2=0.260		pseudo r^2=0.299	
	(n=523)		(n=595)	
Variable	**Odds ratio (95% CI)**	**p value**	**Odds ratio (95% CI)**	**p value**
Number of years since delivery (per year)	N/A	N/A	1.05 (0.75, 1.45)	0.782
Number of subsequent GDM pregnancies (per one pregnancy)	N/A	N/A	0.46 (0.19, 1.11)	0.085
Breastfeeding after delivery	0.63 (0.34, 1.14)	0.126	0.64 (0.22, 1.85)	0.411
Continued breastfeeding on hospital discharge	N/A	N/A	0.35 (0.07, 1.67)	0.186
Breastfeeding for > 1 month	N/A	N/A	2.79 (0.71, 10.90)	0.140

Table 3.8 Predictive factors for the presence of abnormal glucose tolerance up to 5 years post index pregnancy. Two separate models were constructed using logistic regression analysis; the first using variables available at the time of delivery only, and the second using variables from the first model (BMI and age replaced with BMI and age at follow-up), with variables available post-delivery included.

3.2.4 Assessing variables associated with measures of glycaemia at retesting

While we wished to identify those women going on to meet the criteria for AGT following a pregnancy complicated by gestational diabetes, we also wished to assess this relationship using continuous variables. We therefore used multivariable linear regression (ordinary least squares method) to model the relationship between our measured variables and measures of glycaemia at retesting for this study; fasting glucose and HbA1c (n=595). The variables included in the models for each of these, and the accompanying B-coefficients, are shown in table 3.9.

Variable	HbA1c at follow-up r^2=0.318 (n=595)		Fasting glucose at follow-up r^2= 0.387 (n=595)	
	ß-coefficient (95% CI)	**p value**	**ß-coefficient (95% CI)**	**p value**
Fasting glucose on pregnancy OGTT (mmol/L)	0.960 (0.526, 1.394)	<0.001	0.341 (0.274, 0.409)	<0.001
1-hr glucose on pregnancy OGTT (mmol/L)	0.263 (0.122, 0.405)	<0.001	0.020 (0.002, 0.042)	0.077
2-hr glucose on pregnancy OGTT (mmol/L)	0.067 (-0.082, 0.215)	0.378	0.000 (-0.023, 0.023)	0.998
Week of gestation tested for GDM (mmol/L)	-0.117 (-0.189, -0.044)	0.002	-0.019 (-0.030, -0.008)	0.001
Pre-term delivery (number of days)	0.167 (-0.329, 0.663)	0.508	-0.035 (-0.111, 0.042)	0.379
Pregnancy –induced hypertension/pre-eclampsia	-0.323 (-0.988, 0.343)	0.341	-0.083 (-0.186, 0.020)	0.115
Emergency Caesarean delivery	-0.263 (-0.934, 0.408)	0.442	-0.004 (-0.108, 0.100)	0.938
Birthweight (kg)	-0.041 (-0.796, -0.020)	0.039	-0.058 (-0.118, 0.02)	0.060
Insulin use during pregnancy	1.177 (0.411, 1.943)	0.003	0.183 (0.064, 0.302)	0.003
Age at follow-up (years)	0.089 (0.041, 0.136)	<0.001	0.007 (-0.001, 0.014)	0.075
BMI at follow-up (kg/m^2)	0.089 (0.046, 0.131)	<0.001	0.014 (0.007, 0.020)	<0.001
Family history of diabetes	0.487 (0.002, 0.973)	0.049	0.105 (0.030, 0.180)	0.006

Variable	HbA1c at follow-up r^2=0.318 (n=595)		Fasting glucose at follow-up r^2= 0.387 (n=595)	
	ß-coefficient (95% CI)	**p value**	**ß-coefficient (95% CI)**	**p value**
Neonatal unit admission	0.007 (-0.667, 0.680)	0.984	-0.021 (-0.125, 0.084)	0.696
Number of years since delivery	-0.040 (-0.329, 0.248)	0.783	-0.001 (-0.046, 0.044)	0.959
Number of subsequent GDM pregnancies	-0.2782 (-0.989, 0.433)	0.443	-0.061 (-0.171, 0.050)	0.281
Breastfeeding after birth	0.140 (-0.747, 1.027)	0.757	-0.014 (-0.152, 0.124)	0.841
Continued breastfeeding on hospital discharge	-0.875 (-1.96, 0.209)	0.757	-0.072 (-0.024, 0.097)	0.402
Breastfeeding for > 1 month	0.758 (-0.077, 1.594)	0.075	0.091 (-0.039, 0.221)	0.168

Table 3.9. Linear regression models for prediction of HbA1c and fasting plasma glucose (FPG) at retesting

HbA1c at follow-up was associated with insulin use during pregnancy, fasting glucose on the pregnancy OGTT, a family history of diabetes, one-hr glucose value on the pregnancy OGTT, week of gestation tested for GDM, age and BMI at follow-up, and a lower birthweight. FPG at follow-up was associated with fasting glucose on the pregnancy OGTT, insulin use during pregnancy, a family history of diabetes, week of gestation tested for GDM, and BMI at follow-up only.

3.3 Discussion

Our results show that, despite the IADPSG criteria being based on adverse pregnancy outcome, they continue to define a cohort of women at significantly increased future risk of AGT, with over a quarter demonstrating AGT at this relatively short post-partum interval. Given the marked heterogeneity in length of follow-up, demographics, and criteria used for diagnosis among cohorts who have been followed up post-GDM, direct comparison with other studies is difficult. Even looking at cohorts with a similar duration of follow-up (62, 66, 73, 77, 113, 153-155), prevalence rates for AGT vary significantly, from 25%(113) to over 50%(77), with different criteria used and/or different ethnic mixes in each cohort.

As expected, given the lower diagnostic thresholds, those women who meet the stricter IADPSG criteria only are at lower (although still relatively high; prevalence of 14%) risk of AGT at this time interval as compared with those women who also meet the older WHO criteria. Although we are unable to directly compare WHO and IADPSG criteria in the whole cohort, as some women meeting WHO criteria for two-hour glucose only may not have been invited for retesting, we have done so for the women identified during the universal screening part of our study. Therefore, although it has been proposed that women meeting IADPSG criteria are likely to require less intensive follow-up for progression to type 2 diabetes than those meeting the older criteria (151), our figures show that the risks remain high, and given that differentiation of those at lower risk cannot be reliably performed clinically, follow-up should be as frequent as for those women meeting the older criteria.

The factors associated with progression to AGT here are similar to those noted in previous studies- pregnancy OGTT values (fasting glucose in particular)(64-67, 69), elevated BMI(89, 111, 112, 156), and earlier diagnosis of GDM (65, 74, 103, 157-159). Family history of diabetes is less

consistently associated with future AGT, although a relationship has been noted previously(63, 89, 150). Also, age and insulin use during pregnancy are associated with higher HbA1c and FPG at follow-up. All of these factors are routinely clinically available, and point to a higher future risk, but as demonstrated by both our decision tree and logistic regression models, they can still not reliably discriminate between those women who require more frequent follow-up to detect AGT from those who do not. Of note, given the absence of evidence to date on a threshold effect seen when the category of overt diabetes in pregnancy is used, we did not include this category separately in the analysis.

This is a clinically important point- identification of glucose abnormalities prior to progression to type 2 diabetes is important in these women for 2 reasons. Firstly, the Diabetes Prevention Program (47) showed a 71% increased crude incidence rate for women with a history of GDM versus those with no GDM history (38.4% vs. 25.7% at three years post randomisation), despite similar characteristics at baseline, but also showed that treatment with lifestyle or metformin reduced the rate of progression to type 2 diabetes(47). Secondly, detection of AGT in women considering further pregnancy allows timely intervention to attempt to reduce the risk of undiagnosed type 2 diabetes, and the potential foetal complications associated with it(34), presenting for the first time in a subsequent pregnancy.

Interestingly, the findings from our multivariate linear regression model show little difference in the variables associated with FPG, and those associated with HbA1c up to 5 years post-partum. With the introduction of HbA1c as a diagnostic tool for diabetes over recent years, this is of clinical relevance. Despite a lack of direct evidence on the cut-off of HbA1c levels that equate to the risk categories of IFG and IGT, categories of higher risk for HbA1c levels have been proposed(38). We have found that, in this ethnically homogenous population, the variables associated with higher FPG levels at medium-term follow-up are essentially the same as those predicting higher HbA1c values. Interestingly, a lower birthweight is

associated with a higher HbA1c at follow-up, the reason for which is not clear, but could potentially be related to more aggressive treatment of GDM with higher glucose levels during pregnancy, but with a higher risk of later progression. This may be of relevance when deciding on which follow-up tests (FPG, HbA1c, both combined, or the OGTT) are the most useful in women with previous GDM.

A limitation of this study is the relatively low uptake on rescreening (45%). Low uptake on follow-up testing is a common issue among women with previous GDM, and is a potential source of bias. However, comparing those women with IADPSG-defined GDM from the original study who did not attend for follow-up against those who did, the mean age at delivery is similar (32.9 years in the original cohort vs. 34.0 in this study); BMI during pregnancy is similar (30.1 kg/m^2 in the original cohort vs. 31.3 kg/m^2 in this cohort); mean parity (1.22 in the original cohort vs. 1.06 in the current study) is also similar. Also, although 1-hr and 2-hr pregnancy OGTT levels are slightly lower, there is no difference in fasting glucose, which shows a stronger association in both this and other studies examining AGT post-GDM.

In summary, women with IADPSG-defined GDM remain a high-risk group for future AGT, and routinely collected clinical variables cannot reliably distinguish between women who will develop AGT post-GDM, and those who will not. Given the increase in numbers of GDM pregnancies associated with adoption of the IADPSG criteria, further study is essential to both determine the optimal method and frequency of follow-up, a topic we will return to in later chapters. Also, given that lifestyle and metformin have been shown to be effective in preventing progression to diabetes in women with previous GDM, further study is needed to establish how best to deliver such interventions for women with previous GDM. Having determined the prevalence of AGT, and factors associated with it, we then turned to the determination of metabolic risk in this cohort, which we will cover in the next chapter.

Chapter 4- The prevalence of metabolic syndrome and insulin resistance among women with previous IADPSG-defined gestational diabetes

4.1 Introduction

As outlined in the introductory chapter, ever since the first standardised diagnostic criteria were introduced in 1964, the post-partum consequences of gestational diabetes mellitus (GDM) have been extensively described. As well as the original criteria-defining risk of progression to diabetes in later life, other outcomes have been reported. An increased prevalence of metabolic syndrome amongst women with previous GDM is well recognised. Having a history of GDM has been associated with a greater post-partum risk of diabetes (relative risk [RR] of 7.7 vs. women with normal glucose tolerance in pregnancy)(55), increased cardiovascular risk (hazard ratio [HR] 1.71 at 11.5 years)(160), and an increased prevalence of metabolic syndrome (up to 3-fold increase vs. women without GDM at 9 years (161).

Our group has previously shown a prevalence of metabolic syndrome (using Adult Treatment Panel III [ATP-III] criteria) of 10.8% at 12 weeks post-partum in women meeting IADPSG criteria for GDM(150), although this did not differ significantly from those women with NGT in pregnancy using the same criteria (8.2% prevalence; OR 1.12, 95% CI 0.59-2.16).

Although the utility of metabolic syndrome as a clinical entity in itself has been questioned (162), it is beyond doubt that it does, at the very least, represent a clustering of cardiovascular risk factors. A diagnosis of metabolic syndrome is associated with several adverse outcomes: type 2 diabetes, (RR 3.53) (163) increased cardiovascular risk (RR 1.65), (164) and all-cause mortality (RR 1.27) . Given that increased cardiovascular risk has been shown to emerge in women with previous GDM(160) following a very

short post-partum period (11.5 years post-partum), identification of those at risk affords a unique opportunity to intervene in an attempt to ameliorate this excess risk. Adoption of the IADPSG criteria will lead to an increased proportion of pregnant women receiving a diagnosis of GDM, with prevalence as high as 25% among some of the HAPO study centres (37). Therefore, outside of glucose abnormalities, identifying those women who are at increased future cardiovascular risk, particularly in light of both their young age, and short interval between index pregnancy and first event, becomes essential. Given the demonstrated association between metabolic syndrome and cardiovascular disease in the general population, use of the ATP-III criteria shows promise as a clinical tool for defining an at-risk cohort who would benefit from aggressive cardiovascular risk factor management(164). In particular, the metabolic and cardiovascular risk profile beyond the immediate post-partum period of women who meet the new IADPSG criteria remains unclear, and thus, the optimal clinical follow-up strategy cannot yet be determined.

4.2 Methods

4.2.1 Metabolic Syndrome

As outlined in chapter 2 (Methods), we invited 270 women with previous GDM by IADPSG criteria and a randomly selected control group of 388 women known to have normal glucose tolerance by IADPSG criteria for retesting. All were of white European origin. In addition to assessment of glycaemic status, as outlined in the previous chapter, we also sought to determine the prevalence of metabolic syndrome and insulin resistance in this cohort.

For the diagnosis of metabolic syndrome, the National Cholesterol Education Program- Adult Treatment Panel III (NCEP-ATP-III) criteria(165) were used (Table 4.1). These were chosen for several reasons. Firstly, they have been used previously in this cohort, and in other studies from our group, and thus enable direct comparison. Secondly, at least in the

Irish population, they are also the most conservative criteria, in that they show the lowest prevalence of metabolic syndrome when applied to a general population(166). Thirdly, in common with the other definitions of metabolic syndrome in common use, their presence has been found to be associated with an increased risk of adverse outcome(163, 164). Finally, in 2009, the WHO recommended consensus criteria, which are in line with the ATP-III criteria used here(167). In keeping with these consensus criteria, while universal agreement on the most suitable waist circumference cut-off to meet this criterion for a diagnosis of metabolic syndrome is awaited, we have used the higher cut-off of 88cm, which on the basis of the current evidence, appears the most appropriate for a population of European origin. This will lead to a lower prevalence of metabolic syndrome than if the International Diabetes Federation criteria of a waist circumference of over 80cm are used(167).

NCEP-ATP III Metabolic Syndrome criteria (Any 3 of 5 needed for diagnosis)
Waist circumference > 88 cm
HDL-cholesterol < 1.3 mmol/L
Blood pressure ≥ 130/85 mmHg
Serum triglycerides ≥ 1.7 mmol/l
Fasting glucose ≥ 5.6 mmol/L

Table 4.1. Metabolic Syndrome criteria employed for this study
NCEP - National Cholesterol Education Program; ATP-III – Adult Treatment Panel III

4.2.2. Insulin resistance

We also set out to determine the level of biochemical insulin resistance in this cohort. We did this for 2 reasons - firstly, to determine if insulin resistance was significantly different between the 2 cohorts, and secondly,

to determine if biochemical insulin resistance was detected in more women than were identified by the ATP-III criteria.

The HOMA2-IR (**HO**meostatic **M**odel **A**ssessment of **I**nsulin **R**esistance) computer model was used to estimate both insulin resistance and beta-cell function. HOMA2-IR is a computer model derived from the original HOMA model described in 1985 at Oxford by Matthews et al (168). This original, widely used, mathematical model assessed beta-cell function (HOMA-%B) and insulin resistance (HOMA-IR) using basal glucose and insulin, or c-peptide concentrations. This used the equations;

HOMA-IR= (fasting plasma insulin x fasting plasma glucose)/22.5, and
HOMA-%B = (20 x fasting plasma insulin) (fasting plasma glucose -3.5)

where insulin was measured in mU/L and glucose in mmol/l.

This model represents the balance between hepatic glucose output and insulin secretion, and is calibrated to yield normal values of 100% for HOMA-B and 1 for HOMA-IR. HOMA correlates with other measures of insulin resistance such as the euglycaemic clamp (r_2=0.73-0.88) and intravenous glucose tolerance test (r_2=0.63)(147). Given the larger numbers involved, however, once-off measurements are frequently used in epidemiological studies for practical purposes. This model was updated in 2004 to HOMA2-IR(147). This is a computer model, has nonlinear solutions, and incorporates an estimate of pro-insulin, allowing the use of specific insulin assays. It also takes renal glucose losses into account. Given the use of a highly specific insulin assay for this study, the HOMA2-IR was more appropriate for use than the older HOMA model. HOMA2-IR can be used over a glucose range of 2.5- 25 mmol/L, and also allows the use of C-peptide. The creators of the HOMA model recommend the use of C-peptide levels to calculate HOMA2-%B, and insulin levels to determine HOMA2-IR where possible, although this is not absolutely necessary(147). Therefore, for this study, insulin concentrations were entered to determine values for HOMA2-IR where possible, and when insulin levels were

unavailable (n=24), c-peptide concentrations were used to calculate this instead. Values for beta-cell function (HOMA-%B) were calculated using C-peptide concentrations where possible, and insulin levels (n=4) when C-peptide was unavailable. It should also be noted that values obtained from the original HOMA-IR model and the HOMA2-IR computer model are not directly comparable: studies comparing the two models in the same population demonstrate that HOMA2-IR values are lower than those obtained using the older HOMA model (169-171).

Insulin resistance for the purposes of this study was determined to be a HOMA2-IR of greater than 1.8; this value represents the 90th percentile of the reference group. There is no universally accepted cut-off for the diagnosis of insulin resistance using HOMA-IR measurements. One of the more common cut-offs is a HOMA-IR value exceeding the 75th percentile of the HOMA-IR value in the reference group(29, 171-173), which would equate to a HOMA2-IR cut-off just 1.3 for this study. However, this would mean that 25% of a young, healthy population known to have no history of glucose abnormalities prior to this study would be diagnosed with insulin resistance by these criteria. This is somewhat different from the unselected reference groups used in previous work in this area. Therefore, we decided on a more conservative cut-point for insulin resistance as a HOMA2-IR value exceeding the 90th centile of the HOMA2-IR values in the reference population (169) (in this case, women known to have normal glucose tolerance in pregnancy).

4.2.3 Statistical analysis

As outlined in the methods chapter, differences between sample proportions were determined using the chi-square test, and differences between continuous variables were determined using the unpaired two-tailed t-test (parametric) or Kolmogorov-Smirnov test (non-parametric). Also, the Bonett-Price method was used to estimate confidence intervals for the difference between sample medians.

4.3 Results

4.3.1 Baseline characteristics

Of 270 women with previous GDM, 265 had sufficient data available to make or exclude a diagnosis of metabolic syndrome, while of 388 with NGT in pregnancy, 378 had sufficient data to make or exclude a diagnosis of metabolic syndrome. Baseline characteristics for these participants are shown in table 4.2.

4.3.2 Metabolic Syndrome

Metabolic syndrome by ATP III criteria was present in 25.3% of women with previous GDM at a mean of 2.6 years post index pregnancy, compared with 6.6 % of women with NGT in pregnancy ($p<0.001$), yielding an OR of 4.8 (95% CI 2.9, 7.8). This is despite the longer time lapse since the index pregnancy (2.6 vs. 3.3 years) (Table 4.2). The prevalence of each component of metabolic syndrome by glucose status in the index pregnancy (NGT and GDM) is shown in Fig 4.1. Waist circumference of greater than 88cm was the most common metabolic syndrome criterion present in both groups, with 55% of previous GDM and 31% of NGT women meeting the criterion. In addition, when analysed as a continuous variable, waist circumference was the strongest individual factor (correlation coefficient 0.523) associated with a diagnosis of metabolic syndrome.

After adjusting for obesity, an excess of risk for metabolic syndrome in women with previous GDM was still evident. Obese women with GDM were more than twice as likely (54.7% vs. 23.4%, $p<0.001$) to have metabolic syndrome compared to obese women who had previous NGT in pregnancy (Fig 4.2). The prevalence of metabolic syndrome was similar in non-obese women in both groups (5.7% vs. 3.2%, $p=0.148$). After adjusting for a family history of diabetes also, an excess risk for metabolic syndrome was present. 34.7% of women with a history of GDM and a family history

of diabetes met metabolic syndrome criteria vs. 9.5% of women with a family history of diabetes, but NGT during the index pregnancy. Metabolic syndrome was uncommon among women with no family history of diabetes (7.6% of women with a history of GDM vs. 3.7% with NGT in the index pregnancy, p=0.158).

4.3.3 Insulin resistance and beta cell function

Insulin resistance data was available for 256 women (97% of cohort) with previous GDM, and 363 women (94% of cohort) with NGT in pregnancy. The median HOMA2-IR (Table 4.3) differed significantly between the groups- 0.9 (IQR 0.7-1.3) in women with NGT compared to 1.4 (IQR 0.9-2.2) in women with previous GDM ($p<0.001$). Beta cell function (HOMAB%), as measured using the HOMA-2IR computer model (Table 4.3), did not differ significantly between the two groups (median 144 % [IQR 124-168] in women with previous GDM vs. 139 % [IQR 120-161] in women with NGT in pregnancy, p=0.108).

The prevalence of insulin resistance, as defined by a HOMA2-IR >1.8 (Table 4.3, Fig 4.2), in this population was 33.6 % in women with previous GDM, compared to 9.1% in women with previous NGT in pregnancy ($p< 0.001$, RR 3.7, [95% CI 2.6, 5.3]). We log transformed HOMA2-IR also, due to a markedly skewed distribution, and found that this correlated significantly ($r=0.520$, $p<0.01$) with a diagnosis of metabolic syndrome by ATP-III criteria. Of note, as outlined in chapter 2, women who did not attend for this study, but who underwent universal screening in the index pregnancy, and met IADPSG criteria for GDM, were slightly younger at delivery (mean age 33.1 years [SD 5.7] vs. 34.0 years [SD 4.9] p=0.046), while their BMI did not differ from women who attended for testing in this study (31.1 kg/m^2 [SD 6.5], vs. 31.4 kg/m^2 [SD 6.6], p=0.611).

Variable	Previous GDM (n=265)	Previous NGT in pregnancy (n=378)	95% CI for difference between groups	p value for difference between groups
Mean age in years	36.7 (SD 5.0)	37.6 (SD 5.1)	0.1, 1.7	<0.001
Mean number of years since index pregnancy	2.6 (SD 1.0)	3.3 (SD 0.7)	0.6, 0.8	<0.001
Mean BMI in kg/m^2	29.7 (SD 6.9)	26.1 (SD 4.9)	2.6, 4,4	<0.001
Mean HbA1c -DCCT units	5.6 % (SD 0.5)	5.3 % (SD 0.3)	0.2, 0.3	<0.001
Mean HbA1c (mmol/mol)	36.2 (SD 4.8)	33.6 (SD 2.8)	2.0. 3.3	<0.001
Prevalence of risk factors for metabolic syndrome				
Prevalence of abnormal glucose tolerance	26 %	3.4 %	17.2, 28.4	<0.001
Prevalence of family history of diabetes mellitus	65.3 %	50 %	11.4, 26.4	<0.001
Prevalence of obesity (BMI ≥ 30 kg/m^2)	40.2%	16.8%	16.0, 29.9	<0.001

Table 4.2. Baseline characteristics for metabolic syndrome cohort at testing for current study.

SD-standard deviation
GDM - gestational diabetes by IADPSG criteria
NGT- normal glucose tolerance by IADPSG criteria

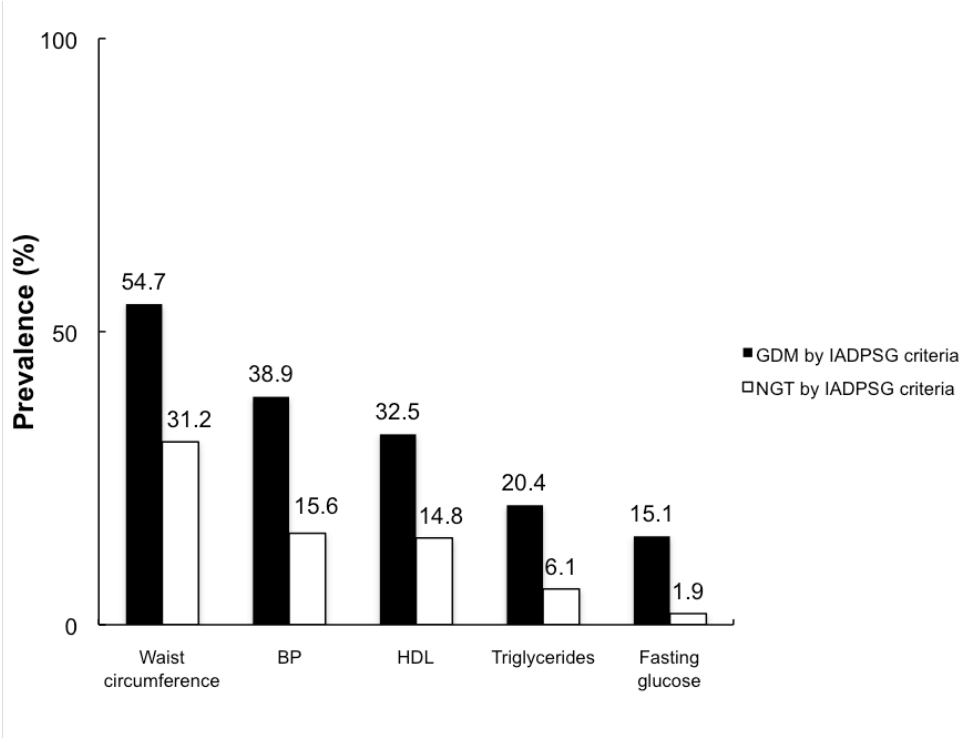

Fig.4.1. Prevalence of each component of metabolic syndrome (using ATP III criteria) by glycaemic status during index pregnancy. Figures represent percentages and p<0.001 between groups for each criterion.

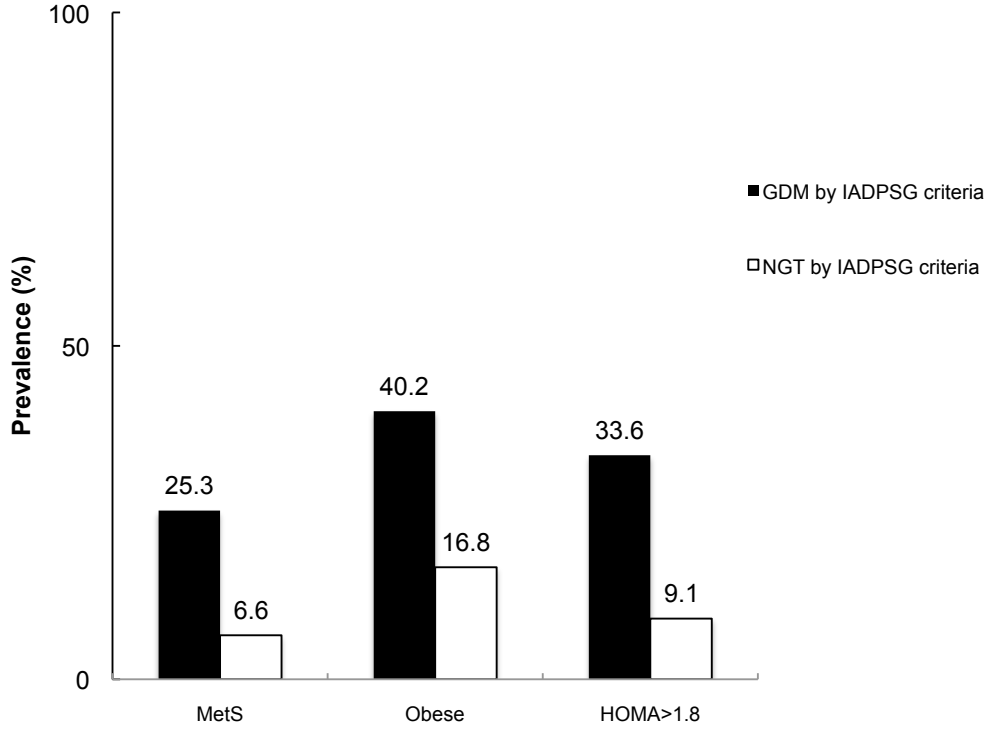

Fig 4.2. Prevalence of metabolic syndrome (ATP-III criteria), obesity (BMI > 30 kg/m^2), and insulin resistance (HOMA2-IR > 1.8) by glycaemic status during index pregnancy. Figures represent percentages, p<0.001 between groups for all characteristics.

Variable	Previous GDM (n=265)	Previous NGT in pregnancy (n=378)	95% CI for difference between groups	p value for difference between groups
Prevalence of Metabolic syndrome / insulin resistance				
Prevalence of metabolic syndrome (ATP- III criteria)	25.3 %	6.6 %	13.0, 24.6	<0.001
Prevalence of HOMA-2 IR > 1.8	33.6 %	9.1 %	18, 31	<0.001
Difference in metabolic syndrome components in women with previous GDM vs. women with previous NGT in pregnancy				
Mean waist circumference (cm)	93.2 (SD 16.3)	84.5 (SD 12.6)	6.4, 10.9	<0.001
Mean fasting glucose (mmol/L)	5.1 (SD 1.0)	4.6 (SD 0.4)	0.3, 0.5	<0.001
Mean HDL-cholesterol (mmol/L)	1.5 (SD 0.4)	1.6 (SD 0.4)	0.1, 0.2	<0.001
Mean SBP (mmHg)	124.4 (SD 15.2)	115.9 (SD 13.3)	6.3, 10.1	<0.001
DBP (mmHg)	73.5 (SD 10.4)	68.7 (SD 9.0)	3.2, 6.3	<0.001
***Median triglycerides (mmol/L)**	1.1 (IQR 0.8- 1.5)	0.8 (IQR 0.6-1.0)	0.2, 0.4	<0.001
Difference in insulin resistance parameters in women with previous GDM vs. women with previous NGT in pregnancy				
***Median HOMA2-IR**	1.4 (IQR 0.9 - 2.2)	0.9 (IQR 0.7 - 1.3)	0.3, 0.7	<0.001
***Median HOMAB%**	144.3 (IQR 123.5 - 168.3)	139.1 (IQR 119.5 – 160.8)	-11.4, 1.4	0.108

Table 4.3: Results from metabolic syndrome and insulin resistance testing
**medians are used due to non-parametric distribution of data.*
SD-standard deviation, IQR-inter-quartile range

Risk factor for GDM	Prevalence in GDM group (absolute numbers)	Prevalence in NGT group (absolute numbers)	p value for difference between groups	95% CI for difference between groups
First-degree relative with diabetes	41.1 % (109/265)	26.5% (100/378)	<0.01	7.3, 22.0
BMI ≥ 30 kg/m^2	83.2% (198/238)	64.2% (204/318)	<0.01˚	11.7, 25.9
Maternal age ≥ 40 yrs	12.1% (32/265)	13.2% (50/378)	0.72	-6.2, 4.2
Long-term steroid use	1.5% (4/265)	0.3% (1/378)	0.17	-0.3, 3.5
Previous baby weighing ≥ 4.5 kg	17.7% (47/265)	11.9% (45/378)	0.04	0.3, 11.7
Diagnosis of Polycystic ovary syndrome	12.8% (34/265)	8.2% (31/378)	0.06	-0.1, 9.8

Table 4.4. Prevalence of risk factors for GDM among women with IADPSG-defined GDM vs. women with NGT by IADPSG criteria.

All participants were of white European origin.
denotes a statistically significant difference between groups (p<0.05)
GDM - gestational diabetes by IADPSG criteria
NGT- normal glucose tolerance by IADPSG criteria

4.3.5 Comparison of women identified by universal or risk factor-based testing

Given that the risk factors for metabolic syndrome and the risk factors used to select women for GDM testing show considerable overlap, we undertook a stratified analysis of those women identified by risk-factor based screening and those identified during universal screening. Of the 265 women with IADPSG-defined GDM, 194 (73%) were identified during the universal screening period. 177 (91%) of these had at least one criterion for risk factor-based screening, as compared with 80% of women with NGT ($p<0.01$). The prevalence of each risk factor among women with GDM compared to women with NGT is shown in table 4.4. Women with GDM showed a significantly higher prevalence of; a family history of diabetes in a first-degree relative (41.1% vs. 26.5% in women with NGT, $p<0.01$); BMI $\geq$ 30 kg/m^2 at the time of testing for GDM (83.2% vs. 64.2%, $p<0.01$); and a history of having delivered a previous baby weighing $\geq$ 4.5 kg (17.7% vs. 11.9%, $p=0.04$). Women with IADPSG-defined GDM identified during universal screening did not differ from those identified by risk-factor based screening in either prevalence of ATP-III-defined metabolic syndrome (26.3% vs. 22.4%, $p=0.63$), or in prevalence of HOMA2-IR>1.8 (34.7% vs. 30.2%, $p=0.54$).

In order to adjust for the effect of risk-factor-based screening, we examined the prevalence of; ATP-III defined metabolic syndrome; its individual components; and HOMA-IR >1.8, in women with IADPSG-defined GDM identified during the universal screening phase. The prevalence of ATP-III defined metabolic syndrome was 26.3% in women with GDM versus 6.3% those with NGT ($p<0.01$). Women with IADPSG-defined GDM also showed a higher prevalence of each of the individual components of ATP-III defined metabolic syndrome; waist circumference >88cm (53.5% vs. 31.4%, $p<0.01$); fasting glucose $\geq$5.6 mmol/L (16.2% vs. 1.9%, $p<0.01$); serum triglycerides $\geq$ 1.7 mmol/l (19.7% vs. 5.5%, $p<0.01$); HDL-

cholesterol < 1.3 mmol/L (30.3% vs. 14.6%, p<0.01); blood pressure ≥ 130/85 mmHg (39.9% vs. 15.4%), and a higher prevalence of HOMA2-IR>1.8 (34.7% vs. 9.2%). When the figures from women identified during the universal screening programme are compared with the those of the entire cohort (as seen in Figs. 4.1 and 4.2), no difference is seen for; a diagnosis of ATP-III- defined metabolic syndrome (p=0.83); any of the individual components of metabolic syndrome (waist circumference; p=0.85, fasting glucose; p= 0.80; triglycerides; p=0.91; blood pressure, p=0.85; HDL-cholesterol, p=0.69); or prevalence of HOMA2-IR >1.8 (p=1.0).

4.4 Discussion

Our results show that despite using the less stringent IADPSG criteria for GDM diagnosis, GDM continues to define a high-risk cohort of women at markedly increased future risk of metabolic syndrome, with 25.3% meeting diagnostic criteria (ATP-III) at a mean of 2.6 years post-partum; a prevalence 3 times greater than that observed in women with NGT during pregnancy. With regard to previous literature on this topic, other studies have examined the prevalence of metabolic syndrome, at varying intervals post-partum, and with older GDM diagnostic criteria. Retanakaran (174) et al examined women with previous GDM at 3 months post partum and found an almost 2-fold increased prevalence (17%) when compared to a control population with NGT in pregnancy (9%). Lauenborg et al (161) also showed a 3-fold increased prevalence of metabolic syndrome at almost 10 years post-partum in women with diet-treated GDM (44%) versus women with NGT (15%). Of note, this study excluded women treated with insulin during pregnancy, and thus the true prevalence may actually be higher. Verma et al (175)demonstrated similar figures to our cohort in a group of 106 women with previous GDM (metabolic syndrome prevalence 27.2%) and 101 controls (metabolic syndrome prevalence 8.2%) who gave birth between 1982 and 1995, and were followed 11 years later. Despite the significantly shorter time elapsed since the index pregnancy (2.6 years) in our IADPSG-defined GDM cohort, and the use of the more conservative ATP-III

criteria(166), we find both a greater than 3-fold elevation of metabolic syndrome in women with prior GDM compared to NGT controls, and a high absolute prevalence of metabolic syndrome, with over 25% of women with previous GDM affected. This is particularly concerning in a population of young women previously considered a lower-risk ethnic group for the development of metabolic syndrome (95). Other studies have attempted to identify factors predicting the later development of metabolic syndrome, and have found that pre-pregnancy obesity(176, 177), weight gain since the index pregnancy(178), family history of type 2 diabetes(179), and unsurprisingly, high fasting glucose levels during pregnancy, contribute(176, 178, 180). However, if the metabolic syndrome is viewed as a cluster of risk factors, associated with future adverse outcomes, rather than a specific pathological entity, the benefit of identifying predictive factors, in the absence of dysglycaemia, in an individual patient is unclear. What is clear however, is that aggressive risk factor management is desirable given the potential for future cardiovascular disease, in both this cohort(160), and the general population with metabolic syndrome(164).

The figures for insulin resistance are also a source of concern. HOMA-IR has been correlated with earlier beta-cell deterioration in the early post-partum period (181). Xiang et al also demonstrated, using detailed physiological techniques (frequently sampled intravenous glucose tolerance test and the hyperinsulinaemic – euglycaemic clamp) in a longitudinal study with up to 12 years follow-up, that lower insulin sensitivity and lower beta-cell compensation for insulin resistance at the baseline assessment were associated with progression to diabetes over the study period (182). With over one-third of our cohort of women with previous GDM by IADPSG criteria displaying values indicative of insulin resistance, this has potentially serious long-term consequences, both for the individual women, and the health services that must plan for their future care.

This effect cannot be entirely explained by obesity alone, although the prevalence of obesity is significantly higher in women with previous GDM (see Fig 4.2). Obesity confers a significant excess risk of metabolic

syndrome in this cohort, but the prevalence of metabolic syndrome still remains double that observed in obese women with NGT in pregnancy. This is also in keeping with recent data from Buchanan et al (183), showing that insulin sensitivity declined faster in women with previous GDM compared to NGT women, although there was no difference in weight change.

Our data on metabolic syndrome and insulin resistance has some important limitations. We are unable to compare the full demographic or metabolic characteristics of this cohort with those of women who did not attend for testing, either in the index pregnancy, or for participation in this study. However, the data we do have available on women who attended for universal screening, but not for retesting in this study shows no significant difference in BMI, blood pressure, or fasting glucose between the 2 groups. Secondly, we are unable to characterise fully the metabolic characteristics of this cohort before the index pregnancy, although data on weight measurements in the index pregnancy were available for the majority of our cohort, and show no significant difference between the groups.

Notwithstanding these limitations, our results demonstrate a significant and clinically relevant difference in metabolic risk factors between the two groups. One in four women with previous GDM meet the diagnostic criteria for metabolic syndrome, while one in three demonstrate biochemical evidence of insulin resistance. This is despite the IADPSG criteria defining what one would assume to be a lower-risk population, due to the lower thresholds and single abnormal value required for diagnosis, and despite these women still being of reproductive age (mean 36.7 years, SD 5.0 years). These figures are similar to a recent meta-analysis(184) examining prevalence of metabolic syndrome post-GDM using various older criteria, which showed an OR of 4.54 (95% CI, 3.78–5.46) for women of European origin, similar to the OR of 4.8 (95% CI 2.9, 7.8) observed in our IADPSG-defined cohort. Efforts focused on lifestyle modification in these high- risk women, in both the preconception and post-partum periods. Such efforts are necessary to try to ameliorate the excess future risk of diabetes, cardiovascular disease, and excess mortality associated with such an adverse

metabolic profile. We will address some potential strategies to detect women most at risk in the next 2 chapters.

Chapter 5 - Use of a diabetes risk score to predict abnormal glucose tolerance up to 5 years post gestational diabetes

5.1 Introduction

The ability to predict which individuals in any given population will develop a disease and which will remain healthy is desirable in all fields of medicine, including diabetes. In the case of women with a previous history of GDM, the ability to predict which women require very close follow-up (i.e. those who will develop pre-diabetes or diabetes), and which women can enter a less intensive follow-up programme, has potentially very significant benefits from both a healthcare and economic perspective. With this in mind, we set out to examine the use of a clinical risk-scoring tool as a convenient, inexpensive way to stratify risk in women with previous GDM.

5.2 History

The 'gold standard' (and until recently, the only accepted method) for the diagnosis of AGT or diabetes is the measurement of plasma glucose. This takes three forms: the 75g oral glucose tolerance test (OGTT), fasting plasma glucose (FPG), or random (i.e. with no regard to the timing of last caloric intake) plasma glucose (RPG) levels. The newer method of diagnosis is using plasma glycosylated haemoglobin levels (HbA1c). Although one or more of these forms of laboratory measurements is absolutely necessary for the diagnosis of diabetes mellitus or AGT(151), each has its own drawbacks as a screening tool. A FPG sample may be inconvenient for both the patient and the practitioner while the OGTT is inconvenient, time consuming, and more costly, as direct, and indirect costs to the woman and health care provider increase.(185) Plasma glucose levels demonstrate random variations, both in populations and within the same patient (186). Glycosylated haemoglobin levels are in their infancy as an approved diagnostic tool, and certain patient conditions, for example, unrecognised anaemia, or haemoglobinopathies, depending on the assay method used, may impact on the result. The significance of an indeterminate

result is also undefined. All of these measurements are, to some degree invasive, and to varying degrees, expensive to perform, particularly at a population level.

The ideal screening test for diabetes, therefore, should be accurate, reproducible, easy to carry out, convenient for the patient and healthcare provider, and cheap. Screening tests should also follow the criteria set out by the WHO (Table 5.1) (Wilson and Jungner criteria(187)).

- The condition should be an important health problem
- There should be an accepted treatment for patients with recognised disease
- Facilities for diagnosis and treatment should be available
- There should be a latent or early symptomatic stage
- There should be a suitable test or examination
- The test should be acceptable to the population
- The natural history of the condition, including development from latent to declared disease, should be adequately understood
- There should be an agreed policy on who to treat as patients
- The cost of case finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole
- Case finding should be a continuous process and not a ‘once and for all’ project

Table 5.1 Wilson and Jungner criteria for disease screening (World Health Organisation criteria)

This led to the interest in developing a risk factor-based diabetes risk score as an assessment tool. The first published risk score was published by the American Diabetes Association in 1993(188) but its applicability to other populations, particularly a Northern European population, was limited(189). Several others followed(190-192), but the first risk factor based score using routinely collected data to achieve wider clinical use was the Cambridge Risk Score(193)(Table 5.2), published in 2000, based on 41 general practices in southern England.

	Variable (x)	Coefficient (ß)
	Constant	-6.322
	Sex (Female)	-0.879
	Antihypertensive therapy prescribed	1.222
	Steroids prescribed	2.191
	Age in years (multiplied by)	0.063
BMI	< 25 kg/m^2	0
	25-27.49 kg/m^2	0.699
	27.5-29.99 kg/m^2	1.970
	30 kg/m^2 or over	2.518
Family history of diabetes	None	0
	Parent or sibling	0.728
	Parent and sibling with diabetes	0.753
Smoking status	Non-smoker	0
	Ex-smoker	-0.218
	Current smoker	0.855

Table 5.2 *The features and coefficients that compose the Cambridge Risk Score to predict type 2 diabetes. These variables are entered into a computer, which then estimates risk using the coefficients detailed above.*

As a cross-sectional tool, this demonstrated an area under the receiver operator curve (AuROC) of 0.8 to identify impaired glucose tolerance (WHO criteria), and at the proposed threshold score of 0.199, the sensitivity was 77%, with 72% specificity, and a likelihood ratio for a positive result of 2.76. When examined subsequently in a general population in Norfolk, again as a cross-sectional tool, the AuROC for HbA1c of ≥6.0% was 0.657 (0.718 for HbA1c≥6.5%). Many risk factor and questionnaire –based screening techniques have since been published (194-198) (199, 200) (148, 199, 201-208) but since the publication of the Cambridge score, the only other score to have entered widespread clinical use is the FINDRISC score and its derivatives (202, 209)

5.3 FINDRISC score

5.3.1 FINDRISC as a predictive tool in longitudinal studies

The development of the FINDRISC (Finnish Diabetes Risk Score) is well described in Lindstrom et al's original paper(148). In brief, for development of the model, a random sample of participants aged between 25 and 64 were randomly selected from the Finnish National Population Register. This sample was stratified to ensure that all age groups and geographical regions were represented. 82% (n=4,746) had completed a medical questionnaire at the time of enrolment. Logistic regression analysis was then carried out to compute the beta-coefficients of easily obtained clinical risk factors, with drug-treated diabetes as an endpoint (identified via the nationwide Social Insurance Institution drug register). This identified the following risk factors for drug-treated diabetes at 10-year follow-up; age (OR 2.6 for age 55 years or over), BMI (OR 2.6 for BMI over 30 kg/m^2), waist circumference (OR 4.16 if ≥ 102cm in men or ≥ 88cm in women), antihypertensive drug therapy (OR 2.04), and a history of high glucose levels (OR 8.5). The 10-year incidence of type 2 diabetes in this cohort was 4.1%. Whole numbers were then assigned to each risk factor, weighted according to the OR derived above, in order to facilitate easier clinical use (See Table 5.3).

Questions detailing physical activity and fruit and vegetable consumption were also added to the scoring tool, despite not adding to the predictive power of the model. Overweight (BMI 25-30 kg/m^2) was also added to the model, as it was felt to be an inevitable step between normal weight and obesity. Once again, however, this was not a statistically significant predictor in multivariate models.

	Variable	Odds Ratio
	Intercept	-5.658 (coefficient)
Age	under 45 years	Reference
	45-54 years	1.92
	55-64 years	2.56
	Antihypertensive therapy prescribed	2.04
Waist circumference	<94cm (men) / <80cm(women)	Reference
	94-101.9 cm (men) / 80-87.99 cm (women)	2.78
	102cm or more (men) / 88cm or more (women)	4.16
	History of high blood glucose	9.61
BMI	< 25 kg/m^2	Reference
	25-30 kg/m^2	1.02
	over 30 kg/m^2	2.55
Family history of diabetes	None	
	Parent or sibling	Not reported
	Parent and sibling with diabetes	
	Physical activity < 4 hrs /week	1.31
	Daily consumption of fruit, vegetables, or berries	1.18

Table 5.3 The features and odds ratios that compose the FINDRISC score. For clinical use, odds ratio were rounded to the nearest whole number, with the exception of 'History of high blood glucose', which was allocated a score of 5, 'Physical activity < 4hrs/week', which was rounded up to a score of 2, and the question 'Family history of diabetes' which was allocated a score of 5 for a first-degree relative, and 3 for a second-degree relative.

The model thus derived (the FINDRISC score) was validated by the same study group in an independent Finnish cohort in 1992 (n=4,615), which had a 5-year incidence of drug-treated diabetes of 1.5%. The FINDRISC performed well in both the derivation (1987) and validation (1992) cohorts to predict drug-treated diabetes, with an area under the ROC curve (AuROC) of 0.85 and 0.87 for the 1987 and 1992 cohorts, respectively(148). Using a cut-off score of ≥ 9, yielded a sensitivity of 78% and specificity of 77% in the 1987 cohort, while the 1992 cohort showed a sensitivity of 81% and specificity of 76%. Therefore, the FINDRISC score was shown to perform well as a prediction tool for future risk of drug-treated type 2 diabetes, in longitudinal studies. Of note, family history data was not available from the original cohorts. However due to the importance of genetic susceptibility, as reflected by family history, in determining risk of developing type 2 diabetes, it was added to the questionnaire for clinical use. Scores of 5 and 3 were chosen, respectively (apparently arbitrarily), for participants with first or second-degree relatives with diabetes(148).

The FINDRISC tool thus created has gained widespread acceptance as a risk-scoring tool since then. It was further validated as a cross-sectional tool; in an Italian population(210), although it did not perform as well, with an AuROC of 0.72 with an endpoint of type 2 diabetes, and 0.67 for an endpoint of AGT; and in a German population(211), with an AuROC of 0.75 for an endpoint of type 2 diabetes. Even more importantly for our cohort, follow-up testing, again under the auspices of the Finnish Diabetes Prevention Study, showed that a higher baseline FINDRISC score predicted those individuals at high-risk of diabetes (in this case, individuals with a diagnosis of impaired glucose tolerance) in which an intensive lifestyle intervention programme was most effective(209).

5.3.2 FINDRISC as a cross-sectional tool for undiagnosed abnormal glucose tolerance

What, however, of the use of the FINDRISC score as a screening tool to identify current undiagnosed diabetes? Cross-sectional performance of the FINDRISC score was also assessed for participants in the original Finnish 1987 and 1992 cohorts who had sufficient data from oral glucose tolerance testing available to diagnose or exclude diabetes. AuROC was 0.80 for both cohorts with a prevalence of undiagnosed diabetes of 3.5% (n=87) in the 1987 cohort, and 5.7% (n=112) in the 1992 cohort. Sensitivity for a cut-off score of ≥9 was 77% and 76% for the 1987 and 1992 cohort respectively, while specificity was 66% and 68% respectively.

Further analysis from the same group examined the usefulness of the FINDRISC score in the cross-sectional setting in a different cohort in 2002, but this time looked at different outcomes, among them AGT(212). FINDRISC performed less well in this setting, with an AuROC of 0.65 for men and 0.65 for women. Similarly, a proposed cut-off score of 11 yielded a poor sensitivity and specificity of 46% and 25%, and 53% and 34% in men and women respectively. Although the authors claim that the FINDRISC performs 'fairly well' in this setting, in fact, these figures would actually appear to limit its clinical usefulness as a tool to predict the presence of AGT in any given patient when used alone. Interestingly, the FINDRISC questionnaire did perform better in detecting metabolic syndrome in the same study, showing an AuROC of 0.73 in men, and 0.75 in women, comparable to its performance in detecting undiagnosed type 2 diabetes in the same population, (AuROC 0.72 and 0.73 in men and women respectively. This study, as with most of the literature examining the use of the FINDRISC score, is based on the use of the WHO criteria for IFG (i.e. using the higher fasting glucose threshold of 6.1 mmol/L. However, it has also been employed as a cross-sectional tool for the detection of abnormal glucose tolerance defined using the ADA criteria(213), with similar AuROC results.

5.4 FINDRISC use in women with previous gestational diabetes

Given the usefulness of the FINDRISC score in detecting those at risk of type 2 diabetes, and, in the cross-sectional setting, detecting undiagnosed diabetes, we sought to evaluate its performance as a cheap, reliable, non-invasive screening test in a population of women with a previous diagnosis of GDM.

Since the introduction of the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria in 2010, they have gained wide acceptance, with influential international bodies such as the WHO and the American Diabetes Association endorsing their use. As we mentioned in our opening chapter, however, the magnitude of future risk of progression to type 2 diabetes, and the attendant risks of macrovascular complications (160), are not clear with IADPSG criteria. Given that up to 25% of pregnancies, depending on the population studied (37), may be complicated by GDM, the optimum follow-up strategy of these women is of the utmost importance from a public health perspective. Any inexpensive, yet reliable clinical aid that could potentially reduce the burden of 1-3 yearly lifelong testing (127) on the patient, healthcare provider, and the healthcare system, is therefore of interest.

With this in mind, we set out to examine the usefulness of the FINDRISC score as a cross-sectional tool to predict current AGT in women with a history of IADPSG-defined GDM. To calculate the FINDRISC score in our cohort, we incorporated all the questions from the available FINDRISC tool (http://care.diabetesjournals.org/content/31/5/857/T1) (209) into our structured questionnaire. This was completed for all patients who presented for follow-up glucose testing as part of this study. Measurements of waist circumference, weight, and height were taken as outlined in Chapter 2. Of note, all women with a history of GDM as defined by IADPSG criteria scored 5 for the question ‘have you ever been found to have high blood glucose?' whether or not they were diagnosed with GDM at the time of the

index pregnancy, while women meeting WHO but not IADPSG criteria for GDM scored 0.

5.5 Results

5.5.1 FINDRISC to predict abnormal glucose tolerance post-partum

Sufficient data to calculate a complete FINDRISC score was available on 269 of 270 women (99.6%) with previous GDM by IADPSG criteria, and all 388 women known to have normal glucose tolerance (NGT) in pregnancy. Table 5.4 shows the median FINDRISC scores in each group, and the percentage of women meeting the criteria for each category of each question in the FINDRISC tool. Significant differences are seen in most, but not all, categories. There was no significant difference in the proportion of overweight participants in each group (33.5% in GDM group vs. 35.1% in the NGT group, p=0.672), or in the proportion of those with those with waist circumference between 80 and 88 cm (24.8% in the GDM group vs. 28.6% in the NGT group, p=0.281). However, a significantly larger proportion of women with previous GDM were obese (39.8% vs. 16.8% in the NGT group, p <0.001), and a larger proportion had a waist circumference of >88cm (55.6% vs. 32% in the NGT group, p<0.001). There was also no significant difference between groups in the proportion of women not eating fruit and vegetables daily (3.3% in the GDM group vs. 2.8% in the NGT group, p=0.441), GDM group vs. 14% in the NGT group, p= 0.082).

Variable	GDM (n=269)	NGT (n=388)	p value for difference between groups
Median FINDRISC score (IQR)	12 (10-16)	5 (3-8)	<0.001
FINDRISC score components			
Proportion with any history of abnormal glucose tolerance	100%	0%	<0.001
Proportion with BMI 25-30 kg/m^2	33.5%	35.1%	0.672
Proportion with BMI >30 kg/m^2	39.8%	16.8%	<0.001
Proportion with waist circumference 80-88cm	24.8%	28.6%	0.281
Proportion with waist circumference >88cm	55.6%	32%	<0.001
Proportion not active for ≥30 minutes per day	10%	14%	0.082
Proportion not eating fruit and vegetables daily	3.3%	2.8%	0.441
Proportion with first-degree family member with diabetes	40.7%	26.3%	<0.001
Proportion with second-degree family member with diabetes	24.5%	23.7%	<0.001
Proportion with history of antihypertensive treatment	3.3%	0.8%	0.018

Table 5.4 Characteristics of participants, including the proportion in highest-risk categories of FINDRISC score parameters

Table 5.5 demonstrates the AuROC using the FINDRISC score to predict AGT at up to 5 years post index pregnancy in women with previous GDM and NGT. The AuROC for women with previous GDM is 0.650 (95% CI 0.577, 0.724), while the AuROC for the cohort as a whole is 0.804 (95% CI 0.760, 0.849). Table 5.6 demonstrates the sensitivity, specificity, negative predictive value, and positive predictive value for specific FINDRISC thresholds of 9 (the cut-off value proposed in the original FINDRISC study) and 11, shown for comparison purposes.

	FINDRISC AuROC at testing for this study **(95% CI)**	**FINDRISC AuROC** using index pregnancy variables only* **(95% CI)**
GDM (n=269)	0.650 (0.577, 0.724)	0.645 (0.571, 0.719)
NGT (n=388)	0.763 (0.666, 0.861)	0.735 (0.634, 0.837)
Whole cohort (n=657)	0.804 (0.760, 0.849)	0.798 (0.753, 0.844)

Table 5.5. Area under the ROC curve (AuROC) values for prediction of abnormal glucose tolerance up to 5 years post-partum using the FINDRISC score in women with IADPSG-defined GDM, and also NGT in pregnancy.
**Due to missing values, AuROC is calculated on 241 women in the GDM group and 327 in the NGT group.*

As can be seen from the above results, the AuROC of 0.763 falls short of the often-quoted standard of 0.800 to be a clinical useful screening tool(214). When a cut-off score of ≥ 9 is used for women with previous GDM, sensitivity is good at 95.7%, although specificity is poor at 21.1%. The same threshold value used applied to the cohort as a whole gives a lower sensitivity of 89.3%, with an improved specificity at 58.1%.

FINDRISC cut-off (no. of women meeting criteria)	**Sensitivity** (95%CI)	**Specificity** (95% CI)	**PPV** (95% CI)	**NPV** (95% CI)
		Women with GDM by IADPSG criteria (n=269)		
≥ 9 (n= 224)	95.7 (88.1, 98.5)	21.1 (16.0, 27.3)	29.9 (24.3, 36.2)	93.3 (82.1, 97.7)
≥ 11 (n=187)	82.3 (72.4, 89.9)	35.2 (28.9, 42.0)	31.0 (24.8, 38.0)	85.4 (76.1, 91.4)
		Women with NGT by IADPSG criteria (n=388)		
≥ 9 (n=91)	57.1 (32.6, 78.6)	77.8 (73.3, 81.7)	8.8 (4.5, 16.4)	98.0 (95.7, 99.1)
≥ 11 (n=28)	28.6 (11.8, 54.7)	93.6 (90.6, 95.6)	14.3 (5.7, 31.5)	97.2 (95.0, 98.5)
		Entire cohort (n=657)		
≥ 9 (n=315)	89.3 (80.9, 94.3)	58.1 (54.0, 62.1)	23.8 (19.4, 28.8)	97.4 (95.1, 98.6)
≥ 11 (n=215)	73.8 (63.5, 82.0)	73.3 (69.5, 76.8)	28.8 (23.2, 35.2)	95.0 (92.6, 96.7)

Table 5.6. Performance of 2 different FINDRISC score cut-offs in women with a history of GDM vs. those with NGT by IADPSG criteria.

PPV-Positive Predictive Value, NPV- Negative Predictive Value

5.5.2 Other applications of the FINDRISC score

We then examined the ability of the FINDRISC score to predict subsequent AGT if performed at the time of diagnosis of GDM (i.e. at 24-28 weeks gestation). This analysis, was, however performed retrospectively, as some of the necessary data was not collected at the time of the current study. Therefore, waist circumference was deemed to be the same at 24-28 weeks gestation as at follow-up for the purposes of this analysis (an assumption

which would not hold true for all patients, of course, but none the less provides some estimate of how the test may perform in pregnancy). Here the AuROC for prediction of future AGT is decreased at 0.645 (95% CI 0.571, 0.719) for women with GDM by IADPSG criteria with BMI measurements available for the index pregnancy (n=241, Table 5.5). Therefore, it is clear that the performance of the test diminishes further when used as a tool during pregnancy in women with GDM to predict later, post partum, progression to AGT.

Finally, we assessed the performance of the FINDRISC as a tool for the prediction of IADPSG-defined GDM at the time of presentation in the index pregnancy, prior to glucose testing. This involved using the same assumptions regarding waist circumference as outlined above, and the same reduced data set due to missing BMI measurements as outlined in Table 5.5 (n=569 for the whole cohort). Again, FINDRISC performance for the prediction of prediction of IADPSG-defined GDM was poor, with an AuROC of 0.681 (95% CI 0.636, 0.725).

5.6 Discussion

Our aim in this part of the study was to evaluate the clinical utility of a diabetes risk score (FINDRISC) in predicting progression to AGT among women with previous GDM at a mean of 2.5 years post index pregnancy. The FINDRISC score is likely to be most applicable to the cohort of patients in our study, by virtue of ethnic mix. As outlined in the methods chapter, our entire cohort is of European ancestry, and this is likely to impact on the risk of diabetes/AGT.

Results from the use of the FINDRISC score to predict AGT in women with a history of GDM at this time interval (mean 2.6 years) are, however, disappointing, with an AuROC of 0.763 (95% CI 0.705, 0.820). Women with previous GDM have high FINDRISC scores (median 12 vs. 5 in those women with NGT), automatically gaining 5 points by virtue of the GDM diagnosis. We see also that there is a higher prevalence of obesity (although

not overweight), and a higher proportion in the highest-risk waist circumference category. It would therefore appear that because these women are at high risk of future AGT in any case, that the FINDRISC tool is not useful to discriminate further those women with previous GDM who are likely to develop AGT. The performance of the FINDRISC tool for the entire cohort analysed together was reasonable with an AuROC of 0.804 (95% CI 0.760, 0.849), and a reasonable sensitivity (89.3%, 95% CI 80.9%, 94.3%), but low specificity at 58.1% (95% CI 54.0, 62.1%). In addition, the FINDRISC tool does not appear to be useful at the time of diagnosis of GDM to predict later AGT (AuROC 0.645, 95% CI 0.571, 0.719), although the assumption we have made regarding waist circumference measurements being similar now and during the index pregnancy limit the conclusions we can draw here.

Ultimately, longer-term follow up of this cohort to detect AGT may yield different figures, as opposed to the cross-sectional approach taken here, but for now, the use of a diabetes risk score to predict progression to AGT in our cohort of women with GDM, at this time interval, cannot be recommended on the basis of our data. Therefore, the emphasis must remain on biochemical testing in women with previous GDM at regular intervals in order to detect progression to AGT, and ultimately, type 2 diabetes. We will discuss this issue further in the next chapter.

Chapter 6 - Comparison of fasting plasma glucose, HbA1c, and oral glucose tolerance test in women with previous gestational diabetes

6.1 Introduction

Since the original O'Sullivan and Mahan diagnostic criteria(215) identified a population of women at a greatly increased risk of progression to diabetes later in life, it has been recognised that lifelong follow-up of these high-risk women is desirable. However, given historically poor post-partum testing rates, there has been long-standing debate regarding the best follow-up method to employ for the routine follow –up of the asymptomatic patient(216-222).

Until 2010, the only way in which a diagnosis of diabetes mellitus could be made was by measuring plasma glucose levels. The 'gold standard' for diagnosis of diabetes outside of pregnancy has for many years been the 75g two-hour oral glucose test (OGTT)(127, 223). This has the advantage of missing no cases (as 'the gold standard' against which other tests are measured), and of being the only method by which impaired glucose tolerance (IGT) can be diagnosed. However, it is inconvenient, requiring an overnight fast, and a minimum two-hour time commitment on the part of the patient. From a healthcare provider perspective, the OGTT involves extra phlebotomy equipment, increased labour costs, and the use of glucose solution to carry out the test(185). Fasting plasma glucose (FPG) has the advantage of requiring only one sample, being more convenient for both the patient and the healthcare provider, and reducing material costs associated with the OGTT. FPG alone is recommended for follow-up by the United Kingdom National Institute for Health and Care Excellence (NICE)(129). However, FPG alone is not sensitive for AGT post-partum, missing up to 83% of cases of IGT and up to 56% of diabetes cases)(87, 156, 224-227). Until recently the OGTT or FPG were the only options available for diagnosis.

However, following the DETECT-2(228) study, an International Expert Committee, the use of HbA1c as an option to diagnose type 2 diabetes(229). This was made feasible due to the increasing adoption worldwide of Diabetes Control and Complication Trial (DCCT)-aligned assays (accomplished via the National Glycohemoglobin Standardization Program) (230), which allowed both meaningful comparison across different sites, and traceability to the assays used in the DCCT and United Kingdom Prospective Diabetes Study (UKPDS). The further alignment of HbA1c assays to a reference method (International Federation of Clinical Chemistry and Laboratory Medicine)(231), with derived DCCT – aligned units has ensured that determining the accuracy and reliability of HbA1c measurements worldwide is possible.

The HbA1c has several advantages over FPG and OGTT as a screening tool for type 2 diabetes. It is convenient, requiring no overnight fast, and requires a single sample only. From a healthcare provider perspective, again, phlebotomy and material costs are reduced in comparison with the OGTT. It also offers an assessment of severity of chronic hyperglycaemia at the time of diagnosis, reflecting glucose levels for the preceding 8-12 weeks.(232) However, although the HbA1c has been validated as a diagnostic tool for type 2 diabetes from the DETECT-2 study (on the basis of prediction of microvascular complications, specifically retinopathy), the cut-offs which correspond to lesser degrees of impaired glucose metabolism as seen on FPG or OGTT (i.e. IFG/IGT) are not clear(233-235). Also, HbA1c results may also be affected by conditions that affect red cell turnover- iron deficiency anaemia (increases values), blood loss, recent transfusion, haemolytic anaemia (decreases values)(236). Variations in HbA1c, independent of glycaemia, have also been reported with increasing age(237) and between ethnicities(238).

Current international guidelines with regard to follow-up of women with GDM vary. All major guidelines agree that the first postpartum evaluation should take place at 6-12 weeks post delivery(127-129, 239). None of the current guidelines recommend the use of HbA1c for diagnosis in this

setting. Given the propensity of the HbA1c to be affected by conditions such as iron deficiency anaemia, and blood loss, both of which are common in the perinatal period, and antenatal glycaemic control, accurate interpretation of the result may be difficult. National Institute for Clinical Excellence (NICE) guidelines recommend evaluation with fasting plasma glucose alone for the first postpartum assessment(129), while the American Diabetes Association and IDF guidelines recommend initial post-partum evaluation with a 75g OGTT(127, 128). The ACOG guidelines cite the OGTT as preferable, but also allow for use of the FPG(239). Our current Irish national guidelines (240) recommend screening with a 75g OGTT at 6-12 weeks post partum.

Guidance on how to follow up these high-risk women after the initial postpartum evaluation varies, however. NICE guidelines recommend the continued use of FPG on an annual basis, the ADA recommend follow-up with either FPG, HbA1c, or 75g OGTT, and recommend follow-up on a 3-yearly basis, reserving annual follow-up for higher risk patients within this subgroup (IFG or IGT). Irish guidelines recommend an annual 75g OGTT. With previous figures from our cohort demonstrating a GDM prevalence of 12.4% during a period of universal screening (with 44% uptake), this means that approximately one in eight pregnant women attending for screening will require lifelong follow-up with an annual 75g OGTT. With approximately 11,000 deliveries per year in the region where our study was carried out(1, 241) this represents a significant financial outlay from a service provision perspective, not to mention a significant time commitment on the part of the women in our population with a history of GDM.

How are these recommendations followed in practice, however? Previous studies from multiple centres have shown unequivocally that uptake rates of post-partum screening, even in the early post-partum period, are low(216-222). Several studies have also attempted to identify issues associated with lower rates of post-partum screening. Broadly speaking, issues can be classified as patient-, provider-, or system – related. Patient issues include time constraints, and competing commitments (e.g. child care), perceived by

women in this population cohort to be a particular issue(242, 243). Also, perception of the future risk of diabetes in this population may not reflect their true risk(244). Healthcare provider issues include physician specialty (219, 245) and lack of clear responsibility for arranging follow-up testing(245). Interventions showing some success include reminders to patients, physicians, or both(246, 247).

In our centre, after the initial post –partum glucose evaluation, all patients attend our clinic for discussion of their results. Those with values diagnostic of diabetes, and those with values diagnostic of IFG or IGT who are considering further pregnancy, are offered follow-up appointments and remain under close follow-up in the secondary care system under the care of a diabetologist. Those women with normal values on OGTT, or those with IFG or IGT, but who are not planning a further pregnancy, are discharged to the care of their general practitioner. A letter is sent to the patient's general practitioner requesting that a 75g OGTT be arranged by the general practitioner on an annual basis. This plan is also outlined to the women on the day of clinic attendance. Although no figures for follow – up testing exist in the Irish population, as outlined above, international screening uptake is low, and strategies to increase this are of great potential clinical benefit. Screening in this high-risk group, as outlined in the opening chapter, provides an opportunity to recognise the onset of type 2 diabetes earlier and institute appropriate care in a timely manner in order to prevent complications. It also allows the recognition of abnormal glucose regulation prior to the development of overt diabetes, at a time when intervention with lifestyle measures or pharmacotherapy may prevent or delay progression to type 2 diabetes (47). Finally, it may reduce the risk of undiagnosed type 2 diabetes presenting in a subsequent pregnancy, representing a lost opportunity for intervention to improve maternal and foetal outcomes (35, 248). One such strategy we wished to evaluate, in order to simplify follow-up, was the role of HbA1c alone, FPG alone, or a combination of both, to predict progression to AGT post gestational diabetes.

6.2 Results

The clinical strategy we envisaged was using either the HbA1c or FPG in order to determine which women would require further testing with 75g OGTT to confirm or out rule AGT.

As outlined in the methods section of chapter 2, all women attending for screening had HbA1c drawn. Of 266 women attending for retesting, 89% (n=237) had a 75g OGTT, while the remaining 11% (n=29: 19 of whom were known to have AGT at their first
post-partum visit) had FPG only. Baseline characteristics are shown in table 6.1. Of the 266 women tested, 15.4% (n=41) were known to have AGT at their first post partum visit (6.8% IFG, 2.6% IGT, 4.5% combined IFG/IGT, 1.5% diabetes mellitus). At retesting, 81.6% (n=217) had normal glucose tolerance, while 18.4% (n=49; 95% CI 14.2 to 23.5) had AGT (IFG, n=30; 11.3%; IGT, n=8; 3%, combined IFG/IGT, n=5, 1.9%; diabetes mellitus, n=6, 2.3%). Of those women meeting IADPSG criteria, but not WHO criteria (n=95), 12% (n=11) had AGT. Baseline characteristics and results at rescreening are summarised in table 6.1.

HbA1c

Table 6.2 shows the test accuracy of HbA1c at defined thresholds for predicting AGT by ADA criteria. Using the recommended ADA HbA1c cut-off for high-risk individuals of 39 mmol/mol (5.7%) yielded a sensitivity of 45% (95% CI 32 to 59), specificity of 84% (95% CI 78 to 88), NPV of 87% (95% CI 82 to 91), and PPV of 39% (95% CI 27 to 52). ROC curve analysis for HbA1c to predict any AGT gave an AuROC of 0.742 (95% CI 0.663 to 0.821).

Characteristic	Total cohort n=263 (SD)	NGT n=215 (SD)	Abnormal glucose tolerance	P value for difference	95% CI for difference
Age (Years)	36.6 (5)	36.6 (5)	36.9 (5.1)	0.700	-1.3, 1.9
Time since delivery (Years)	2.6 (1.0)	2.6 (1.1)	2.7 (1.0)	0.298	-0.2, 0.5
BMI (kg/m^2)	29.7 (6.9)	29.1 (6.8)	32.4 (6.8)	0.002	1.2, 5.5
Median HbA1c (range) (mmol/mol)	36 (61)	35 (19)	38 (54)	<0.001	1.5, 4.5
Fasting glucose (mmol/mol)	5.1 (1)	4.8 (0.4)	6.1 (1.7)	<0.001	0.9, 1.9
2 hour glucose (mmol/L)	5.6 (1.8)	5.2 (1)	8.0 (3)	<0.001	1.7, 3.8
Waist circumference (cm)	93.3 (16.4)	91.8 (16.3)	100.1 (15.5)	0.001	3.2, 13.4
Abnormal glucose tolerance at 12 weeks (%) (n)	16 (43)	N/A	N/A	N/A	N/A
Abnormal glucose tolerance at rescreening (%) (n)	19 (49)	N/A	N/A	N/A	N/A

Table 6.1 Characteristics of women with previous IADPSG-defined GDM at enrolment in the current study
IADPSG- International Association of Diabetes and Pregnancy Study Groups

HbA1c (mmol/mol)	**No. of women meeting criteria*** (%)	**No of cases missed ****	**Sensitivity** (%- 95%CI)	**Specificity** (%- 95%CI)	**PPV** (%- 95%CI)	**NPV** (% - 95%CI)
32	244 (93)	1	99 (97, 100)	8 (5, 13)	20 (15, 25)	95 (75, 99)
33	229 (87)	2	96 (87, 99)	15 (11, 20)	21 (16, 26)	94 (81, 98)
34	203 (77)	3	94 (83, 98)	27 (21, 33)	23 (17, 29)	95 (86, 98)
35	174 (66)	7	86 (73, 93)	38 (32, 45)	24 (18, 31)	92 (85, 96)
36	139 (53)	10	80 (66, 89)	53 (47, 60)	28 (21, 36)	92 (86, 96)
37	108 (41)	14	71 (58, 82)	66 (59, 72)	32 (24, 42)	91 (85, 95)
38	79 (30)	22	55 (41, 68)	76 (70, 81)	34 (25, 45)	88 (83, 92)
39	57 (22)	27	45 (32, 59)	84 (78, 88)	39 (27, 52)	87 (82, 91)
40	36 (14)	31	37 (25, 51)	92 (87, 95)	50 (35, 66)	86 (81, 90)
41	26 (10)	34	31 (20, 45)	95 (91, 97)	58 (39, 75)	86 (81, 90)
42	17 (7)	36	27 (16, 40)	98 (95, 99)	77 (53, 90)	86 (81,90)
43	12 (5)	38	23 (13, 36)	100 (97, 100)	92 (65, 99)	85 (80, 89)
44	9 (3)	40	18 (10, 31)	100 (98, 100)	100 (70, 100)	85 (80, 89)
45	8 (3)	41	16 (9, 29)	100 (98, 100)	100 (68, 100)	84 (79, 88)
46	7 (3)	42	14 (7, 27)	100 (98, 100)	100 (65, 100)	84 (79, 88)
47	6 (2)	43	12 (6, 24)	100 (98, 100)	100 (61, 100)	83 (78, 87)
48	6 (2)	43	12 (6, 24)	100 (98, 100)	100 (61, 100)	83 (78, 87)

Table 6.2 Performance of different HbA1c cut-offs to predict abnormal glucose tolerance post IADPSG-defined GDM
IADPSG- International Association of Diabetes and Pregnancy Study Groups

FPG (mmol/L)	No. of women meeting	No of cases missed**	Sensitivity (%- 95%CI)	Specificity (%- 95%CI)	PPV (%- 95%CI)	NPV (% - 95%CI)
5	139 (53)	7	84 (71, 92)	61 (55, 67)	33 (25, 42)	94 (89, 97)
5.1	108 (41)	8	82 (69, 90)	68 (62, 74)	37 (29, 46)	94 (89, 97)
5.2	91 (35)	8	82 (69, 90)	76 (70, 81)	44 (34, 54)	95 (90, 97)
5.3	69 (26)	8	82 (69, 90)	87 (81, 90)	58 (46, 69)	95 (91, 98)
5.4	60 (23)	9	80 (66, 89)	90 (85, 94)	65 (52, 76)	95 (91, 97)
5.5	48 (18)	9	80 (66, 89)	96 (92, 98)	81 (68, 90)	95 (92, 97)
5.6	39 (15)	9	80 (66, 89)	100 (98, 100)	100 (91, 100)	96 (92, 98)
5.7	36 (14)	12	74 (60, 84)	100 (98, 100)	100 (90, 100)	94 (90, 97)
5.8	32 (12)	16	65 (51, 77)	100 (98, 100)	100 (89, 100)	93 (89, 95)
5.9	28 (11)	20	57 (43, 70)	100 (98, 100)	100 (88, 100)	91 (87, 94)
6	26 (10)	22	53 (39, 66)	100 (98, 100)	100 (87, 100)	90 (86, 93)
6.1	25 (10)	23	51 (38, 64)	100 (98, 100)	100 (87, 100)	90 (85, 93)

Table 6.3 Performance of different FPG cut-offs to predict abnormal glucose tolerance post IADPSG-defined GDM
IADPSG- International Association of Diabetes and Pregnancy Study Groups

Fasting Plasma Glucose

Using the ADA high-risk criterion for FPG ≥ 5.6 mmol/L to identify any degree of AGT, sensitivity was 80% (95% CI 66 to 89), specificity was 100% (95% CI 98 to 100), NPV was 96% (95% CI 92 to 98), and PPV was 100% (95% CI 91 to 100). The characteristics for different cut-offs of FPG when used to screen for abnormal glucose
tolerance are summarised in table 6.3. ROC curve analysis examining the ability of FPG alone to predict IGT (i.e. to predict a 2 hr plasma glucose of ≥ 7.8 mmol/l showed an AuROC of 0.609 (95% CI 0.438 to 0.779).*HbA1c and FPG combined*

The above results show suboptimal performance using HbA1c or FPG alone to detect AGT in this cohort. We therefore used defined cut-offs of a combination of HbA1c and FPG to identify higher-risk women who should proceed to confirmatory glucose testing with a 75g OGTT. Women were classified as meeting the criteria if they met either the specified HbA1c or the FPG value. We calculated the negative predictive value (NPV), positive predictive value (PPV), sensitivity and specificity for each defined cut-off of a combination of HbA1c and FPG values. Results are shown in table 6.4.

6.3 Discussion

As can be seen from table 6.4, the performance of the 'test' (HbA1c and FPG combined) varies significantly with regard to the cut-offs chosen. Taking the most lenient cut-offs illustrated above as a starting point, it is immediately evident that using this approach significantly reduces the number of women proceeding to further testing. At cut offs of HbA1c of 37 mmol/mol and FPG of 5.3 mmol/L, less than half of women proceed to further testing. Sensitivity is good at 92% (95% CI 81, 97%), with an excellent NPV of 97% (95% CI 93, 99%). Specificity is reasonable at 50%. Raising the cut-offs to improve specificity reduces sensitivity accordingly, for example at HbA1c of 5.6 mmol/l with HbA1c of 42, sensitivity drops to 84% (95% CI 71, 92%), with a specificity of 98% (95% CI 95, 99%).

Choosing where the thresholds should be placed is therefore not a matter of simply determining the best figures, but rather balancing the clinical risks of missed diagnoses versus the implications of screening large proportions of the population at risk.

Therefore, our decision was to place the threshold for further testing at an HbA1c level of 39 mmol/mol (derived DCCT 5.7%) and an FPG of 5.6 mmol/L. This identifies 90% (95% CI 78, 96%) of women with AGT, with a satisfactory NPV of 97% (95% CI 94, 99%), and a reasonable specificity of 84%. PPV is 56% for these cut-offs. The proportion of women in this cohort meeting these cut-offs is low at 30%, reducing the number of women going on to 75g OGTT by over two-thirds.

However, in meeting these criteria, these women have already been identified as high-risk by either their FPG of ≥ 5.6 mmol/L (28%, n=22), their HbA1c ≥ 39 mmol/mol (51%, n=40), or both (22% n=17). Given that the ADA guidelines specify both the FPG cut-off value of 5.6 mmol/L and HbA1c cut-off of 39 mmol/mol as high risk categories, approach to these women is similar from a clinical perspective – these women should all be offered preventative measures with lifestyle intervention where available, while pharmacotherapy with metformin could be considered for selected patients(47). A 75g OGTT would appear to add little to the management of these women. There is insufficient evidence to determine the optimal approach for those women (44%, n=35) who meet HbA1c criteria, but in whom the 75g OGTT is not diagnostic. Our suggested approach to longer-term follow-up in women with previous GDM is outlined in figure 6.1 Also, employing this new approach (requiring only a single blood draw) to identify those higher risk women who should proceed to a 75g OGTT would reduce the number of OGTTs performed by almost seventy percent. At an estimated cost of Euro 35,200 per 1000 women tested using 75g OGTT(185), this new screening regime would reduce the cost of OGTT screening to Euro 10,560 although this would of course by offset by the cost of measuring HbA1c and FPG in each patient.

FPG (mmol/L)	**HbA1c** (mmol/ mol)	**No. of women meeting criteria*** (%)	**Cases miss-ed****	**Sensitivity** (95%CI)	**Specificity** (95%CI)	**PPV** (95%CI)	**NPV** (95%CI)
5.3	**37**	128 (49)	4	92 (81, 97)	50 (44, 57)	35 (27, 44)	97 (93, 99)
5.6	**37**	117 (45)	5	90 (78, 96)	66 (59, 72)	38 (29, 47)	97 (92 99)
6.1	**37**	112 (43)	10	80 (66, 89)	66 (59, 72)	35 (27, 44)	93 (88, 96)
5.3	**39**	96 (37)	4	92 (81, 97)	76 (70, 81)	47 (37, 57)	98 (94, 99)
5.6	**39**	79 (30)	5	90 (78, 96)	84 (78, 88)	56 (45, 66)	97 (94, 99)
6.1	**39**	68 (26)	16	67 (53, 79)	84 (78, 88)	49 (37, 60)	92 (87, 95)
5.3	**42**	73 (28)	7	86 (73, 93)	86 (80, 90)	58 (46, 68)	96 (93, 98)
5.6	**42**	45 (17)	8	84 (71, 92)	98 (95, 99)	91 (79, 97)	96 (93, 98)
6.1	**42**	32 (12)	21	57 (43, 70)	98 (95, 99)	88 (72, 95)	91 (87, 94)
5.3	**48**	70 (27)	8	84 (71, 92)	87 (81, 90)	59 (47, 69)	96 (93, 98)
5.6	**48**	40 (15)	9	82 (69, 90)	100 (98, 100)	100 (91, 100)	96 (92, 98)
6.1	**48**	26 (10)	23	53 (39, 66)	100 (98, 100)	100 (87, 100)	90 (86, 94)

Table 6.4 Performance of different cut-offs for a combination of HbA1c and FPG to predict abnormal glucose tolerance post IADPSG-defined GDM
IADPSG- International Association of Diabetes and Pregnancy Study Groups

It should be noted that there are significant limitations to this data, however, for two main reasons. Firstly, this is cross-sectional data on a cohort, identified retrospectively as having GDM by IADPSG criteria during the index pregnancy. This approach is now in the process of being prospectively evaluated across our own centres. Secondly, as outlined in previous chapters, the cohort consists of women of white European origin only. The effect of ethnicity on HbA1c levels is well documented (238). Therefore, our recommendation would be to continue annual 75g oral glucose tolerance tests in women who are not of white European ancestry. Another limitation in our study is that 11% of women (n=29) did not undergo a repeat OGTT for this study, but had fasting glucose levels only. Removing these women from the analysis, and taking the proposed cut-offs of HbA1c of 39 mmol/mol (5.7%) and fasting glucose of 5.6 mmol/L, the sensitivity drops slightly to 85% (95% CI 70, 94), with a slightly increased specificity of 86% (95% CI 80, 90). PPV and NPV are similar at 50% (95% CI 38, 62) and 97% (95% CI 94, 99) respectively. However, given that those women who had fasting glucose levels only represent a higher-risk group (69% of these 29 women were known to have AGT at their first post-partum visit), we feel the best approach is to include these women in the analysis. This approach would also be similar to that taken in the clinical management of these women. Also, the majority of the women invited for retesting for this study (89%) underwent an OGTT. Offering the option of just a single blood draw for FPG and HbA1c (or even a single non-fasting sample for HbA1c alone) may well have a significant effect on the relatively low (58%) uptake of our offer of retesting. Using an FPG cut-off of 5.6 mmol/L as part of the high-risk criteria to detect abnormal glucose tolerance, will of course, by default, generate a high AuROC for this test, either alone (0.873, 95%CI 0.795, 0.950), or in combination with HbA1c (0.888, 95%CI 0.817, 0.919), when 5.6 mmol/L is the cut-off used to define abnormal glucose tolerance (ADA criteria). However, this does in fact, represent the clinical approach taken. The issue of missing diagnoses of post-partum abnormal glucose tolerance and diabetes when a 2-hr values is not used is well described, with up to 83% of cases of IGT, and up to 56% of cases of diabetes missed(249). This approach aims to minimise the number of diagnoses missed when an

OGTT is not employed.

The results of this study, interestingly, are similar to those in recent papers by Megia et al(250) and Picon et al(251) who employ similar approaches to predict abnormal postpartum glucose tolerance, albeit describing a lower cut off; HbA1c of 37mmol/mol (5.5%; Megia). There are several important differences between the studies, however. Our study shows a sensitivity of 90% versus 82% (Megia) and 83% (Picon), while we demonstrate a higher NPV (97%) versus Picon et al (85%). This is a key difference when designing a pragmatic retesting program for women with previous GDM. For these purposes, a higher sensitivity and NPV are desirable, and in this cohort, do not result in an unacceptable increase in confirmatory testing; the proportion of women meeting HbA1c/ FPG criteria, and therefore requiring confirmatory testing, is 31% as compared to 29% in Megia et al and 47% in Picon et al. Both Megia and Picon's studies involve higher risk cohorts, using the National Diabetes Data Group criteria for GDM as opposed to the newer, more stringent, IADPSG criteria, and accordingly, demonstrate a higher prevalence of AGT using OGTT; 45.9% in Picon et al and 27.8% in Megia et al. This is despite a shorter interval to postpartum retesting- 3 months (Megia) and one year (Picon) versus 2.6 years in our cohort. Other important differences include the ethnic composition of the cohorts- our cohort is 100% white European, compared to 8.5% of Megia et al's cohort being comprised of ethnic minorities (predominantly Arabic and Hispanic). Given the relatively low GDM prevalence of 12.4% in previous studies from our group (compared to the 17.8% across all HAPO centres(37)), the overall burden of follow-up testing, although significant, may be less than other centres. Also, the HbA1c assay used in the Megia and Picon studies is DCCT aligned, while our assay is fully metrologically traceable to the newer IFCC standard. Another study by Kim et al(252)in 54 women with a history of GDM further demonstrates the limitations of using HbA1c in isolation to predict AGT, showing an AuROC of 0.76 for AGT on OGTT, and a sensitivity of 65% and specificity of 68% for predicting AGT when an HbA1c cut-off of greater than or equal to 5.7% (39 mmol/mol) is used. Although this study demonstrates that our approach is clinically feasible, a

randomised controlled trial to compare uptake and effectiveness of the various testing modalities would be useful.

In summary, the combination of HbA1c and FPG measurements to predict AGT shows results superior to either one used alone. 90% of women with AGT are identified using cut offs of greater than or equal to 39 mmol/mol for HbA1c or 5.6 mmol/l for fasting plasma glucose, while reducing the number of OGTTs performed by over two-thirds. Therefore, in the absence of high-quality evidence to determine the optimal interval for repeated retesting of glucose status in women meeting IADPSG criteria for GDM, follow-up with HbA1c and FPG would appear to be a promising strategy.

This proposed approach may have a significant economic and social benefit from both a patient and healthcare provider perspective if it is shown to be effective on prospective analysis. However, as cost-effectiveness is a key element of any proposed screening programme, detailed economic evaluation, including not just the comparative cost of the test themselves, but detailed estimation of the potential consequences of missed cases (e.g. unrecognised type 2 diabetes presenting for the first time in a subsequent pregnancy, with the associated increased risk of congenital malformation or other adverse pregnancy outcome)(48) will be necessary to provide an accurate cost-benefit analysis.

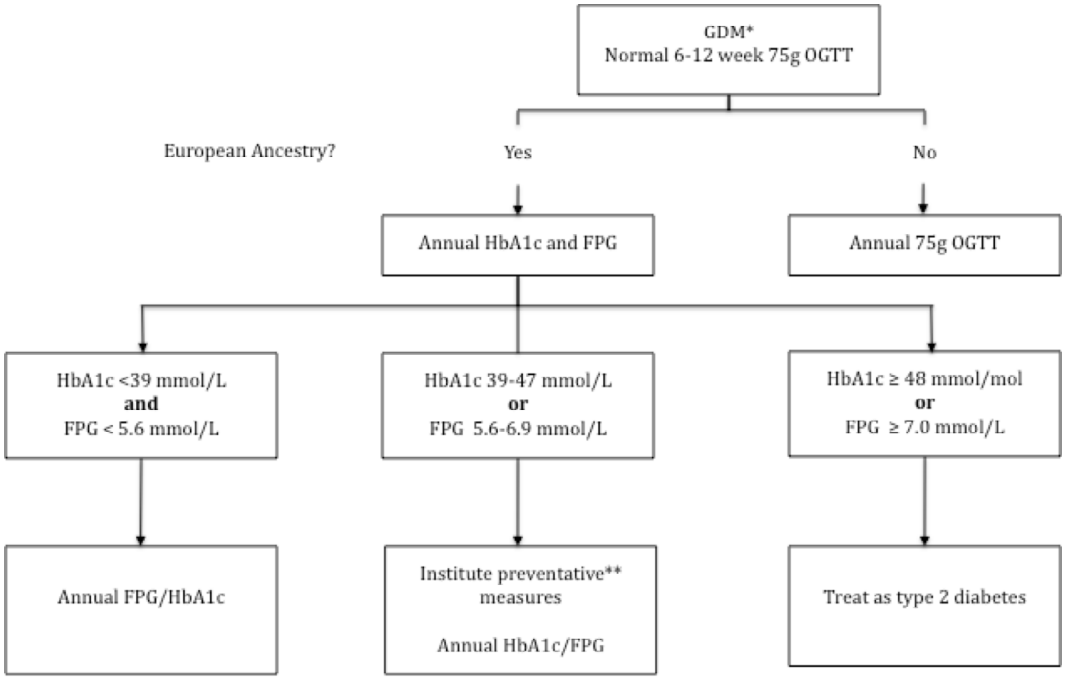

Fig 6.1 Proposed algorithm for longer-term follow-up of women with GDM by IADPSG criteria and normal test at first post-partum visit.

**GDM by International Association of Diabetes and Pregnancy Study Groups criteria*
*** Lifestyle measures +/- metformin*

Chapter 7- Conclusions

With this study, we set out to determine the prevalence of AGT in a cohort of women meeting the new International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria for the diagnosis of gestational diabetes (GDM). We have demonstrated that these criteria continue to define a high-risk cohort of women for progression to type 2 DM. Given the heterogeneity of studies looking at progression to AGT and diabetes post-GDM, it is not possible to directly compare this risk to older studies using the National Diabetes Data Group (NDDG), Carpenter-Coustan, and World Health Organisation (WHO) criteria. It appears (and would seem logical) that it the risk in women diagnosed using the IADPSG criteria is slightly less than in those using the older criteria. However, the optimal method and frequency of follow-up is unknown. Further long-term follow-up of women meeting IADPSG criteria will be essential in order to determine a robust, practical, and broadly applicable follow-up strategy for these women.

Also, we have established that none of our current approaches (predictive models based on index pregnancy factors, diabetes risk scoring tools) allow us to discriminate between those women who will develop diabetes and those who will not. As it stands then, the current principle of close (1-3 yearly) follow-up with biochemical assessment of glycaemic status remains the cornerstone of assessment. Our proposed approach of using HbA1c and FPG to carry out annual retesting shows some promise, but must be validated prospectively, in our population, and others, particularly those with a broader ethnic mix, to which this strategy might not be suited(238).

We have also demonstrated a worryingly high prevalence of metabolic syndrome, and an even higher prevalence of biochemical insulin resistance. On the basis of currently available data regarding cardiovascular risk in women with a history of GDM(160), this is of grave concern. Again, prospective follow-up of this cohort will be essential to determine the risk to

these women beyond their childbearing years, and to determine if biochemical insulin resistance correlates well with future risk.

However, although the accurate determination of future risk is vital, particularly when planning a long-term follow-up strategy, it is of course the treatment of the individual patient that is the major concern.

Sufficient high quality-evidence, from a small number of randomised clinical trials demonstrates that lifestyle intervention, or treatment with metformin reduces the risk of progression to diabetes(47). However, these studies involved long and intensive lifestyle intervention programmes. Thus examining different methods of translating these findings into routine clinical practice are important(253, 254), and a randomised trial examining the feasibility and effectiveness of a 12-week cardiovascular health programme is currently underway in our centre.

The data from these studies will be helpful as we plan future care. However, long- term follow-up of large cohorts of women with IADPSG-defined GDM is necessary to determine, among other things the lifetime risk of progression to diabetes among these women, and effective methods of delaying or halting progression. With up to one in 4 pregnancies being complicated by GDM with the IADPSG criteria in use, timely, effective intervention on a worldwide scale is necessary to help relieve the economic and healthcare burden associated with this.

Chapter 8 –References

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Appendix 1

Study Protocol

CLINICAL RESEARCH PROTOCOL

Protocol Number: 1

Study Title: Conversion to Type 2 Diabetes and Pre- Diabetes 1-5 years following Gestational Diabetes (GDM) in an index pregnancy.

Short Title: **Diabetes Conversion Study**

Version: 1

Date : **March 18th 2011**

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INVESTIGATOR PROTOCOL AGREEMENT PAGE

I, the undersigned, am responsible for the conduct of the trial at this site and agree to the following:

- I understand and will conduct the trial according to the protocol, any approved protocol amendments, ICH GCP and all applicable regulatory authority requirements and national laws.
- I will not deviate from the protocol without prior written approval from the Institutional Review Board or Independent Ethics Committee, except where necessary to prevent any immediate danger to the subject.
- I have sufficient time to properly conduct and complete the trial within the agreed trial period, and I have available an adequate number of qualified staff and adequate facilities for the foreseen duration of the trial to conduct the trial properly and safely.
- I will ensure that any staff at my site(s) who are involved in the trial conduct are adequately trained regarding the protocol and their responsibilities.

Signed

_____ _____

Principle Investigator Galway Date

SYNOPSIS

Study Title:
Study Title: Conversion to Type 2 Diabetes and Pre Diabetes 1-5 years following Gestational Diabetes Mellitus in an Index Pregnancy.

Background
Gestational Diabetes is common in Ireland. Through the ATLANTIC DIP (Diabetes in Pregnancy) programme and using the new evidence based IADPSG criteria, a prevalence of 12.4% was found through universal screening using a 75g OGTT in Pregnancy. In the ATLANTIC DIP programme, the researchers rescreened these women with GDM in an index pregnancy up to 6 months post partum. Type 2 diabetes and pre-diabetes (impaired fasting glucose (IFG) and impaired glucose tolerance (IGT)) was present in 18% of women compared to 2% of controls. The factors associated with the development of diabetes were family history of diabetes, body mass index (BMI), the level of fasting blood glucose on OGTT in index pregnancy and the need for insulin in the index pregnancy. Metabolic syndrome was present in a significant number. The ATLANTIC DIP programme has shown that obesity is a big problem in women of reproductive age in Ireland and significantly affects pregnancy outcome and contributes to the development of GDM. When analysing the factors associated with persistent dysglycaemia postpartum, BMI is a big contributor. Type 2 Diabetes is on the increase. Along the Irish Atlantic seaboard 25% of women presenting with pre-gestational diabetes in pregnancy have Type 2 disease. Women with Type 2 Diabetes are usually older, more obese and of non-Caucasian background and a significant number are from a lower socio-economic group.

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Study Objectives
The **overall aim** of this proposed study is to identify the rate of conversion to Type 2 Diabetes and Pre-Diabetes (IFG and IGT) 1-5 years following GDM in the index pregnancy
Primary objective.
The primary objectives of this study are as follows:

- To identify the pregnancy related factors associated with the development of diabetes/pre diabetes 1-5 years after index pregnancy.
- To identify the number of women with Metabolic Syndrome (MetS) and insulin resistance (IR) 1-5 years post partum.
- To identify the prevalence of overweight (OW) and obesity (OB) in this population.
- To make recommendations on a regional pragmatic recall/screening programme**.**

Endpoints:

- To make recommendations on a regional pragmatic recall/screening programme**.**

Study Design:

This is a single arm prospective follow up study of women 1-5 years following Gestational Diabetes Mellitus (GDM)in the index pregnancy.
Study Population: Women resident in the HSE West with previous GDM between 01.01.2006 and 31.12.2010 will be invited to participate.
Inclusion Criteria: All women with IGT/GDM in the index pregnancy. **Exclusion criteria** Women who are known to have developed diabetes at the initial post partum screen will not be invited for the OGTT but will be included in the data collection.
Data Analysis and Statistics: There are 11,000 deliveries annually in HSE West in 5 centres and we have identified prevalence of 12.4% for GDM using IADPSG criteria (12). Already these women have been rescreened after the index pregnancy and we have found that 18% of women continue to have a glucose problem within the first 6 months (20, 21). Since 2006, 1129 patients with GDM/IGT are on our database, approximately 250 per year. Those with persistent established diabetes/glucose intolerance (18%, N = 203) from the initial screen will not be rescreened but will be included in the analysis. Of the remaining 926 we would anticipate a 70% acceptance rate for a rescreen, similar to that seen in ATLANTIC DIP programme (N = 648) (20).

1. Introduction

1.1 Background Information

Gestational diabetes mellitus (GDM) has been defined by the World Health Organization (WHO) as glucose intolerance resulting in hyperglycaemia of variable severity, with onset or first recognition during pregnancy (1). There is a paucity of robust evidence relating to the prevalence of GDM in the international literature (2). Prevalence estimates have been affected by the wide range of definitions and test criteria used and vary according to region and ethnic group (3). However, GDM undoubtedly affects significant numbers of pregnancies. A 2008 editorial in the Lancet suggested that incidence of GDM is spiraling, with the condition affecting up to 5% of all pregnancies (4). American Diabetes Association guidelines estimate that some 7% of all pregnancies are affected by GDM, resulting in more than 200,000 cases in the USA annually(5). Several factors have been shown to be associated with an increased risk of GDM. Increasing age, previous GDM (associated with a recurrence rate in subsequent pregnancies of 30-84%(3), previous IGT, pre-pregnancy obesity, a previous macrosomic baby (>4.5 kg), a family history of diabetes (first-degree relative with diabetes), and particular ethnic origins are generally accepted as risk factors. It has long been recognised that overt diabetes diagnosed during pregnancy is associated with significant perinatal morbidity such as macrosomia and neonatal hypoglycaemia (6) but the association between lesser degrees of glucose intolerance and morbidity had not been definitively proven until the publication of the hyperglycaemia and adverse pregnancy outcomes (HAPO) study (7). The authors found significant continuous associations between maternal glucose and adverse pregnancy outcomes for mother and offspring at levels below those generally accepted as indicative of overt diabetes (7) Based on the results of HAPO and other studies, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recently issued a consensus statement on new criteria for the diagnosis GDM(8). Accurate up-to-date data on the incidence of hyperglycaemia in pregnancy in Ireland were lacking prior to 2007. It has been over a decade since Irish prevalence figures of overt diabetes of 2.7% were published (9). In the intervening time, the prevalence of obesity (10) and immigration of non-Caucasian individuals (11) both risk factors for diabetes mellitus (DM) - have increased significantly. As a result we carried out a study to determine the true prevalence of GDM using the new IADPSG criteria and quantify the associated adverse maternal and neonatal outcomes. Using universal screening of a regional population 12.4% of women had GDM using IADPSG criteria. GDM pregnancies were associated with statistically significant increased incidence of adverse maternal (pregnancy-induced hypertension, polyhydramnios and caesarean section) and neonatal (prematurity, large for gestational age, neonatal unit admission, neonatal hypoglycaemia and respiratory distress) outcomes. The odds ratio for the development of these adverse outcomes remained significant after adjustment for maternal age, body mass index and non-Caucasian ethnicity (12).

Long term issues

Infant

A number of European studies have considered outcomes for children of GDM pregnancies in the longer term. Finnish longitudinal research identified significant associations between maternal GDM and adolescent manifestations of metabolic syndrome and increased BMI at age 16 (13). In a large Swedish study the hospital care of children born to 82,684 GDM women was compared to 1,213,957 controls by linking the Swedish Medical Birth Registry with the Hospital Discharge Registry Children of GDM women. GDM children had a statistically significant increase in hospitalizations, evident at least up to 10 y of age. Reasons for hospitalizations were neurological/developmental disorders (increased risk compared with non-diabetic pregnancy offspring with a significant odds ratio of 1.36), malformations (OR 1.23), infections (OR 1.20) and accidents (OR 1.14)(14).

Mother

Women with GDM have a significantly increased risk of developing Type 2 diabetes postpartum. A systematic review and meta-analysis of international studies involving over 650 000 women concluded that women with gestational diabetes are seven times more likely to develop type 2 diabetes than women with normoglycaemic pregnancies (RR 7·43, 95% CI 4·79—11·51) (15). A cumulative incidence of type 2 diabetes of about 50% at 5 years is reported in women who have had GDM(16). Many European studies support this. A Finnish study that monitored 174 women with GDM during pregnancy and up to five years subsequently found that 30% developed DM and 51% some form of glucose intolerance. HbA1c and fasting glucose levels in the upper normal range during pregnancy were found to be predictive, as were a family history of diabetes or GDM in a previous pregnancy (17). An Austrian prospective cohort study that included 672 women with GDM also determined that previous GDM as well as obesity independently predicted diabetes postpartum with odds ratios of 4.4 and 4.0 respectively(18). A UK retrospective follow up of 189 GDM women 4.5 years after childbirth also identified HbA1c and glucose levels at GDM diagnosis predictive of future DM as well as pregnancy weight and age. Although subsequent DM was more common amongst South Asians, it was found to represent a significant risk regardless of ethnicity (19). In the ATLANTIC DIP programme we have rescreened women with GDM in an index pregnancy up to 6 months post partum. We found that Type 2 diabetes and pre-diabetes (impaired fasting glucose (IFG) and impaired glucose tolerance (IGT)) were present in 18% of women compared to 2% of controls. The factors associated with the development of diabetes were family history of diabetes, body mass index (BMI), the level of fasting blood glucose on OGTT in index pregnancy and the need for insulin in the index pregnancy (20). We also found that the metabolic syndrome was present in a significant number (21).Type 2 Diabetes is on the increase. Along the Irish Atlantic seaboard 25% of women presenting with pre-gestational diabetes in pregnancy have Type 2 disease (22). Women with Type 2 Diabetes are usually older, more obese and of non-Caucasian background and a significant number are from a lower socio-economic group (23-28). The ATLANTIC DIP programme has shown that obesity is a big problem in our area and significantly affects pregnancy outcome (29). When analysing the factors associated with persistent dysglycaemia within 6 months of delivery, BMI is a big contributor (20)

1.2 Justification of the project.

The rates of Diabetes in Pregnancy (GDM) are rising .It affects at least 1 in 10 women in the West of Ireland. Risk factors include increased weight, older age, and family history of diabetes. After the pregnancy the problem persists in 18% of the women. 82% of women return to normal. GDM is a risk factor for the development of Type 2 Diabetes in later life and rates as high as 50% in the 5 years after GDM have been quoted in the literature. There is no information in Ireland about Type 2 Diabetes 1-5 years after GDM.

In this piece of research, it is proposed to identify how common Type 2 Diabetes is 1-5 years after GDM by rescreening a population previously screened as part of our ATLANTIC-DIP (Diabetes in Pregnancy) programme.
The risk factors that contribute to the development of Type 2 Diabetes will be identified.
This information is important to plan an effective recall system for early detection of diabetes. It will also allow clinicans to target the risk factors implicated and thus try to prevent diabetes.

1.3 Study Rationale.

It is proposed to identify how common Type 2 Diabetes is 1-5 years after GDM by rescreening a population previously screened as part of our ATLANTIC-DIP (Diabetes in Pregnancy) programme.
Risk factors that contribute to the development of Type 2 Diabetes will be identified
Preventative measures may then be developed following identification of risk factors.

2. Study Objectives

The overall aim of the study is to identify the rate of conversion to Type 2 Diabetes and Pre-Diabetes (IFG and IGT) 1-5 years following GDM in the index pregnancy.
The study has a number of objectives**:**

2.1 Primary objective.

- To determine the prevalence of diabetes/pre-diabetes 1-5 years following GDM in an Irish Population.
- To determine the prevalence of Metabolic Syndrome and Obesity in this population.
- To establish how common is insulin resistance in women who continue to have normal glucose tolerance?
- To establish factors that predict the development of dysglycaemia 1-5 years post partum.
- To identify the pregnancy related factors associated with the development of diabetes/pre diabetes 1-5 years after index pregnancy.
- To identify the number of women with Metabolic Syndrome (MetS) and insulin resistance (IR) 1-5 years post partum.

- To identify the prevalence of overweight (OW) and obesity (OB) in this population.
- To make recommendations on a regional pragmatic recall/screening programme

2.2 Study Endpoints

The study endpoint is to determine the prevalence of diabestes and prediabestes in women following gestational diabetes and to make recommendations on a regional pragmatic recall/screening programme

3. Study Design

This is a single arm prospective follow-up study, 1-5 years following GDM in the index pregnancy.

There are 5 participating sites for recruitment to the study: including University College Hospital Galway, Portiuncla Hospital Ballinasloe, Mayo General Hospital Castlebar, Sligo General Hospital, Letterkenny General Hospital .The co-ordinating centre will be at University College Hospital Galway (UCHG) in conjunction with the academic departments of medicine and obstetrics at National University of Ireland Galway (NUIG).

DIAMOND is a Diabetes Clinical Management system at each centre.
Patients who were pregnant during the past 5 years and developed gestational diabetes during their index pregnancy will be identified using the Diamond system.
These patients will be invited to participate in the study.
Following patient information and consent to participate, the patient will make one study specific visit where blood samples and body measurements will be taken.
Based on their Oral Glucose Tolerance Test (OGTT) result, patients will be categorised as one of the following:

1) **Negative**: fasting blood glucose (FBG) < 5.6 mmol/l, 2h blood glucose <7.8 mmol/l;
2) **Impaired fasting glucose** (IFG) FBG >5.6 <7mmol/l; 2h blood glucose <7.8mmol/ls
3) **Impaired glucose tolerance** FBG 5.6-7mmol/l; 2h blood glucose 7.8-11.1mmol/l
4) **Diabetic** FBG >7mmol/l; 2h blood glucose >11.1mmol/l

The Metabolic Syndrome will be diagnosed according to the ATPIII criteria, if the patient has three or more of the following (a) Abdominal obesity, waist circumference >88cm (b) hypertriglyceridaemia >1.69mmol/l (c) Low HDL < 1.29mmol/l (d) High blood pressure >130/85 mmHg (e) fasting glucose >6.1mmol/l.

Based on the results, the participant will be followed up clinically by her local endocrinologist or she will be advised to attend their GP annually for follow up.

4. Selection of Study Population.

4.1 Study Population

Women who reside in the HSE West with previous GDM between 01.01.2006 and 31.12.2010 will be invited to participate.
Participants will be selected from all ATLANTIC –DIP network sites

4.2 Inclusion criteria

- All women with IGT/GDM in the index pregnancy.

4.3 Exclusion criteria

- Women who are known to have developed diabetes at the initial post partum screen will not be invited for the OGTT but will be included in the data collection.

4.4 Withdrawal Criteria

Subjects who consent to the study have the right to withdraw consent at any time .Their data will be removed from the database and will be destroyed in line with data protection act.

5. Assessments and Procedures

Following informed consent women will have a 2hour 75g Oral Glucose Tolerance Test (OGTT) according to the inclusion/exclusion criteria with measurement of glucose and insulin fasting and 1 and 2 hours following the glucose load.
Blood will also be drawn for the measurement of C-peptide, total cholesterol (TC), high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol and triglycerides (Tg) and glycated haemoglobin (HbA1C) at the fasting point.

Each woman will have a weight (kg) height (m2) and body mass index (BMI) calculated and waist circumference (cm) measured .

Based on their OGTT test result, patients will be categorised as one of the following:

1) Negative: fasting blood glucose (FBG) < 5.6 mmol/l, 2h blood glucose <7.8 mmol/l;
2) Impaired fasting glucose (IFG) FBG >5.6 <7mmol/l; 2h blood glucose <7.8mmol/ls
3) Impaired glucose tolerance FBG 5.6-7mmol/l; 2h blood glucose 7.8-11.1mmol/l
4) Diabetic FBG >7mmol/l; 2h blood glucose >11.1mmol/l

The Metabolic Syndrome will be diagnosed according to the ATPIII criteria, if the patient has three or more of the following (a) Abdominal obesity, waist circumference >88cm (b) hypertriglyceridaemia >1.69mmol/l (c) Low HDL <

1.29mmol/l (d) High blood pressure >130/85 mmHg (e) fasting glucose >6.1mmol/l.

6. Risks

There are no potential adverse effects other than possible effects of phlebotomy such as pain or bruise at the site of puncture and a possible, although low, risk of infection.

7. Benefits

Based on the results of the blood tests and the study measurements, there may be modification management of the patients to reflect in as much as possible the best standard of care. If results show that the patient has developed type 2 diabetes or is at increased risk, then the participant will be monitored and managed clinically.

There may be a benefit to the community. This research may contribute to develop predictive tests for type 2 diabetes in women who had gestational diabetes.

8. Ethics and Regulatory Considerations

Ethics approval will be obtained for this study from the ethics committees governing the participating institutions. All study procedures will be conducted in line with the International Conference of Harmonisation, Good Clinical Practice Guidelines, and the Declaration of Helsinki (Appendix 2).
As this is a translational study, there is Regulatory Authority oversight.

9. Patient Registration

9.1 Informed Consent and Patient Registration.

Before a subject can participate in the study, she must give written informed consent. The informed consent process will be in accordance with the Declaration of Helsinki and any local regulatory requirements.
Subject Information Leaflets/Informed Consent Forms and any subsequent changes must be approved by the approving Ethics Committee.

Potential patients will be screened and enrolled on the clinical study on the basis of the inclusion/exclusion criteria specified in the protocol. If the patient is eligible for inclusion and patient consent is obtained ,the investigator will registration the patient onto study. Patients enrolled onto study will be assigned consecutive numbers starting at subject # 001

10. Data Protection

10.1 Subject Confidentiality

The Investigator must ensure that the subjects' anonymity is maintained. On documents subjects should not be identified by their names, but by their assigned

identification number and initials. If subject names are included on any copies of documents the names (except for initials) must be obliterated and the assigned subject numbers added to the documents.
The Investigator should keep a separate log of subjects' identification numbers, names, addresses, telephone numbers and hospital numbers (if applicable).
Documents such as signed Informed Consent Forms, should be maintained in strict confidence by the Investigator

10.2 Data Handling and Record Keeping

Data required according to this protocol is to be recorded on an electronic case report form (CRF). All entries on the CRF, including corrections will be made by an authorised member of the investigator's staff. The original CRFs will be reviewed by the principle investigator .The principle investigator will retain the electronic case report form records at site. Data will be entered into the study database and verified through the use of programmed edit checks for accuracy and completeness. The corrected data and a complete audit trail of corrections will be retained.

10.3 Publication of study findings and use of information

All information regarding the study data or results supplied to the investigator is privileged and confidential information. The investigator agrees to use this information to accomplish the study and will not use it for other purposes.

11. Indemnity

This is an academic led prospective follow-up study is covered in Ireland under the Clinical Indemnity Scheme.

12. Statistical Methods

12.1 Sample Size and Statistical Analysis

There are 11,000 deliveries annually in HSE West in 5 centres and we have identified prevalence of 12.4% for GDM using IADPSG criteria (12). Already these women have been rescreened after the index pregnancy and we have found that 18% of women continue to have a glucose problem within the first 6 months (20, 21). Since 2006, 1129 patients with GDM/IGT are on our database, approximately 250 per year. Those with persistent established diabetes/glucose intolerance (18%, N = 203) from the initial screen will not be rescreened but will be included in the analysis. Of the remaining 926 we would anticipate a 70% acceptance rate for a rescreen, similar to that seen in ATLANTIC DIP programme (N = 648) (20).

The data collected for the study will be processed in the DIAMOND information system. The crude and age-specific prevalence percent (and corresponding 95% confidence interval) of glucose dys-regulation and metabolic syndrome in those women who had developed gestational diabetes during the index pregnancy will be calculated. Classification Trees will be used to gain insight into the underlying structure allowing an identification of potentially useful predictors (and their interactions). Logistic regression and log-linear models (using variable selection

techniques as appropriate) will be used to model the relationships between continued impaired glucose tolerance and metabolic syndrome and identify those risk factors that contribute to the development of Type 2 Diabetes. All analyses will be carried out using SPSS (Version 18.0) and R (version 2.11). The level of statistical significance for the study will be set at 0.05.

13. Laboratory Methods:

This can be seen in appendix 1.

14. Study oversight

14.1 Study Management Group

A Study Management Group will be formed comprising the Principal Investigator, co-investigators, trial statistician, scientists and any study members with a specific interest. The Study Management Group will be responsible for the day to day running and management of the study and will liaise approximately every six months but may convene more often by other means if required.

14.2 Study Monitoring

An internal audit of a sample of the data will be conducted annually against the source documentation at each site to assure quality.
100% review of the patient consents will be verified against assigned subject numbers. This review will be conducted by a member of the research team locally who is not involved in the day to day management of this project.

14.3 Changes in the conduct of the study and Protocol Amendments

All protocol amendments will be submitted to the Ethics Committee for review and approval before implementation.

14.4 Premature Termination Of Study Sites

It is not anticipated that there will be premature termination of study . However, if this occurs, then the data will be analysed and results circulated to team members.
The Ethics Committee will be notified of premature termination and the reasons for termination.

14.5 Archiving

All data will be stored safely and securely following study closure for a period of 7 years.

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16. Appendix 1

Blood Specimen Management

HaemoglobinA_{1c} (HbA_{1c})

The Menarini HA8160 automated haemoglobin (Hb) analyser will be employed to measure HbA_{1c} in ethylenediaminetetraacetic acid (EDTA) anticoagulated whole blood. The method principle is reverse phase cation exchange chromatography and calibration is in accord with IFCC (International Federation of Clinical Chemistry) standardisation (30). The High Pressure Liquid Chromatography (HPLC) column contains micro particles of a copolymer of methylacrylate ester. Haemoglobin fractions pass via the column and are retarded to varying degrees dependent on their molecular size and electrostatic attraction to the particles. A sequential washing with different ionic strength solutions releases the various haemoglobin fractions in turn. As each fraction is eluted from this column, it passes through an optical flow cell. Dual wavelength absorbance at 415 nm and 500 nm is monitored and presented as a series of "peaks" on a printed chromatogram. The area of each peak is calculated and printed in the individual chromatogram report and the stable HbA_{1c} is expressed as mmol of HbA_{1c} per mol of Haemoglobin and derived Diabetes Contol and Complications Trial (DCCT) units (%). Metrological traceability permits HbA_{1c} to be reported in IFCC units (mmol/mol) and derived DCCT/ National Glycohemoglobin Standardization Program (NGSP) units (%) using the IFCC-DCCT/NGSP master equation (NGSP = 0.09418*IFCC (mmol/mol) + 2.152) which links IFCC results to clinically meaningful HbA_{1c} results from the DCCT and the United Kingdom Prospective Diabetes Study (UKPDS) (31).

Total Cholesterol:

The Roche Modular Analytics <P> Chemistry Systems will be employed to measure Cholesterol in serum. The test principle is enzymatic colorimetric assay. Cholesterol is determined enzymatically using cholesterol esterase and cholesterol oxidase. Optimization of ester cleavage (> 99.5%) allows standardization using primary and secondary standards and a direct comparison with the Centre for Disease Control and Prevention (CDC) and National Institute of Standards and Technology (NIST) reference methods (32-34). Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. The free cholesterol that is produced together with the free cholesterol that was initially in the sample, is then converted by oxygen with the aid of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide created forms a red coloured product by reacting with 4-aminophenazone and phenol under the catalytic action of peroxidase. The colour intensity is directly proportional to the concentration of cholesterol and is determined photometrically.

Triglyceride:

The Roche Modular Analytics <P> Chemistry Systems will be employed to measure Triglyceride in serum. This method is based on the work by Wahlefeld (35) and uses a lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red coloured product (Trinder endpoint reaction). The colour intensity is directly proportional to the concentration of cholesterol and is determined photometrically (36).

High-Density Lipoprotein Cholesterol (HDL)

The Roche Modular Analytics <P> Chemistry Systems will be employed to measure HDL in serum. Direct determination of HDL-cholesterol in serum is achieved using polyethylene glycol (PEG)-modified enzymes and dextran sulfate. When cholesterol esterase and cholesterol oxidase enzymes are modified by PEG, they show selective catalytic activities toward lipoprotein fractions, with the reactivity increasing in the order: LDL < VLDL ≈ chylomicrons < HDL(36,37). The cholesterol concentration of HDL-cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups. Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to cholestenone and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino-antipyrine and sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HSDA) to form a purple-blue dye. The colour intensity of thisdye is directly proportional to the cholesterol concentration (38). This direct HDL-cholesterol assay meets the 1998 National Institutes ofHealth (NIH) / National Cholesterol Education Program (NCEP) goals for acceptable performance. The results of this method correlate with those obtained by precipitation-based methods and also by an ultracentrifugation method (36,38)

Insulin:

The Roche E170 Modular Analytics Immunoassay System will be used to measure serum Insulin. The insulin assay is a non-competitive immunochemiluminescent assay based on the use of two monoclonal antibodies directed at different epitopes of the human insulin molecule. The serum is first incubated with a biotinylated monoclonal insulin-specific antibody, and a monoclonal insulin-specific antibody labeled with ruthenium- sandwich assay design. Following this incubation, streptavidin-coated microparticles are added to the reaction mixture, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with the reagent ProCell. Application of a voltage to the electrode then induces chemiluminescent emission that is measured by a photomultiplier. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode (36-38)

C-Peptide:

The Roche E170 Modular Analytics Immunoassay System will be used to measure serum C-Peptide. The Roche C-peptide assay is a 2-site immunometric (sandwich) assay using electrochemiluminescence detection. Patient specimen, biotinylated monoclonal C-peptide specific antibody, and monoclonal C-peptide-specific antibody labeled with ruthenium react to form a complex. Streptavidin-coated microparticles act as the solid phase to which the complex becomes bound. Voltage is applied to the electrode inducing a chemiluminescent emission from the ruthenium, which is then measured against a calibration curve to determine the amount of C-peptide in the patient specimen(36, 38).

Glucose:

The Roche Modular Analytics <P> Chemistry Systems will be employed to measure plasma glucose. The hexokinase method is used and is based on the work of Schmidt, Peterson and Young, is a recognized reference method (36). In this enzymatic assay,

Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate. This reaction is coupled to a second, where Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during this reaction is directly proportional to the glucose concentration and can be measured photometrically at 340nm. (36, 38)

Insulin Resistance and Sensitivity:-

Homeostatic model assessment (HOMA) will be employed in this study for assessing insulin sensitivity (%S) and ß-cell function (%B) from basal (fasting) glucose, insulin and C-peptide concentrations. To assess absolute resistance or ß-cell function, the corrected nonlinear (computer) model will be used, as this has been recalibrated in line with current insulin assays and extended to permit the use of C-peptide if required (39). The computer model gives a value for insulin sensitivity as HOMA2-%S (where 100% is normal) (39).

17. Appendix 2 :Declaration of Helsinki

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.
   The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must

be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

8. In medical practice and in medical research, most interventions involve risks and burdens.
9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.
10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

1. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.
2. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
3. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
4. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.
5. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee,

especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.

6. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.
7. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
8. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
9. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
10. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
11. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
12. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
13. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
14. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After

ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

15. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.
16. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.
17. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.
18. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
19. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.
20. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be

published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

1. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.
2. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:
    - The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
    - Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.
3. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.
4. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.
5. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

18. Appendix 3 :Patient Information Leaflet and Consent.

PATIENT INFORMATION SHEET

Study Title: Conversion to Type 2 Diabetes and Pre Diabetes 1-5 years following Gestational Diabetes Mellitus in an Index Pregnancy.

Principal Investigator: Professor Fidelma Dunne Department of Endocrinology, University College Hospital, Galway and Head of the School of Medicine, National University of Ireland Galway.

We are inviting you to take part in a research project. Before you agree to take part in the study you must understand why we are doing the study and what will be expected of you if you agree to take part. We are providing you with this information sheet to explain the study to you but if you have any questions about the study after reading this sheet please ask us.

What is the purpose of this study?
The purpose of this study is to determine how common type 2 diabetes is in women who had gestational diabetes during pregnancy and also to determine when is the most likely time that diabetes will occur following pregnancy.

Why have I been chosen?
You have been asked to participate in this study because you developed gestational diabetes during your pregnancy.

Do I have to participate?
Participation in this research project is entirely voluntary. You do not have to take part. If you choose not to take part this will not affect the care that you receive from your medical team. If you do agree to take part you will be asked to sign a consent form. However you are free to withdraw from the study at any stage despite signing this form and you are not obliged to give a reason for your withdrawal.

What is required of me if I take part?
Following consent onto study, you will be asked to fast from 12 mid night and attend the clinic or hospital .Blood samples will be taken to measure blood levels of cholesterol and proteins that may indicate presence of risk factors that contribute to diabetes.
An oral glucose tolerance test will also be done. For this, you will have a blood sample taken, you will then be given a glucose drink .You will have a blood sample taken to measure glucose and insulin levels in your blood one hour and two hours after taking the glucose drink .
You will also have your weight, height and waist circumference measured.
These tests will be complete after one visit to the hospital.

Why do I have to have a blood test?
The blood samples will need to be taken in order to carry out the research to determine

Are there any risks involved in participating in this study?
No risk is associated to the participation in this study, except the ones associated with the blood test, such as pain or bruise at the site of puncture and a possible, although low, risk of infection.

What will I do if I have more questions?
If you have questions about any aspect of this study you should contact the Researcher or Principal Investigator who is named at the end of this sheet.

Will my participation in this study be confidential?
Your general details will be collected from your hospital chart and will be entered onto the study database. This information will be stored anonymously and securely by the researchers. During the course of the study authorised personnel may review your medical chart and collected data to assist with the research study all of them have a duty of confidentiality to you as a research participant.

Can I withdraw from this study.
It is up to you to decide whether or not to take part. If you do, you will be asked to sign a consent form. You are still free to withdraw at any time and without giving any reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive from health professionals.

What will happen to any blood samples I give?
The analysis of blood samples will be carried out at the University Hospital Galway.

What will happen to the results of the research study?
The aim would be to publish the results of the research in relevant medical journals and if necessary present the results at suitable medical meetings. At no time will you be identified in any report or publication.

Who has reviewed the study?
This study has been reviewed by the Galway Regional Hospitals Research Ethics Committee.

A copy of this information sheet and consent form will be given to the patient to keep and a copy will be kept in the patients file.

For additional information now or in the future please contact:
Principal Investigator's Name: Professor Fidelma Dunne
Address: Department Endocrinology, University College Hospital, Galway
And Head of the School of Medicine, National University of Ireland Galway,
Phone Number: 091 524222

Consent Form

Study Title: Conversion to Type 2 Diabetes and Pre Diabetes 1-5 years following Gestational Diabetes Mellitus in an Index Pregnancy.

Principal Investigator: Professor Fidelma Dunne Department of Endocrinology, University College Hospital, Galway and Head of School of Medicine, National University of Ireland Galway.

Please read each sentence and initials each box if you agree.

		Initial
1.	I am an adult and of my own freewill and not under duress have agreed to take part in this study.	
2.	I confirm that I have read and understood the Patient Information Sheet Version 1 Date 18th March 2011 for the above study and have been given a copy to keep. The information has been fully explained to me and I have been able to ask questions. I understand why the research is being done and any risks involved.	
3.	I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason and without my rights being affected in any way	
4.	I agree to have blood samples taken from me for research purposes.	
5.	I agree that at the time of delivery samples of blood can be taken from the umbilical cord.	
6.	I understand that relevant sections of my medical notes and data collected during the study may authorised individuals such as those from the University College Hospital Galway, the Clinical Research Facility Galway and the National Centre for Biomedical and Engineering Science. I give permission for these individuals to have access to my records.	
7.	I agree that information about me that is relevant to the above study may be held on a secure confidential database in accordance with the Data Protection Acts (1988 & 2003).	

_____	_____	_____
Name of Subject	Signature of Subject	Date
_____	_____	_____
Name of Witness if applicable	Signature of Witness if Applicable	Date
_____	_____	_____
Name of Person taking consent	Signature of Person taking Consent	Date

Appendix 2

Patient Information Leaflet

What happens if I do not participate?
If you do not participate, your future medical care will not be affected in any way.

Confidentiality
Your identity will remain confidential, and your name or any identifying details will not be published, or disclosed to a third party. Study information will be kept in a secure location in National University of Ireland Galway/University College Hospital Galway.

Costs/Payments

Is the study safe and ethical?
The NUI/UCH Galway Ethics committee have reviewed and approved this study on ethical grounds.

Contact Details
Professor Fidelma Dunne,
Diabetes Day Centre,
University Hospital Galway,
Galway.
Tel (091)
Email:

Should you agree to participate, we will arrange a suitable appointment. Should you have any further questions regarding the study, please contact us as outlined above. We will be in telephone contact over the coming days to arrange an appointment also. Please bring this sheet with you on the day of the test.

Consent

I have read the participant information as outlined above, and have had the opportunity to ask questions and discuss the study. I understand that I am free to withdraw from the study at any time, with no negative consequences to myself, and agree to participate in the study.

Signed

Print name

Date

Appendix 3

Data collection form

Appendix 3- Data collection form

THE CONVERSION STUDY

Patient Study number:	
Control (C)/ Patient (P)	C ☐ P ☐
Consent form signed:	Yes ☐ No ☐
Date of study:	/ /
Anthropometric:	
Blood Pressure: Measure 1: Measure 2:	 SBP DBP SBP DBP
Weight (kg):	
Height (m):	
Waist Circumference (cm):	
Neck Circumference (cm):	
Medical History:	
1st degree relative with DM:	Yes ☐ No ☐
2nd degree relative with DM:	Yes ☐ No ☐
Previous baby >4.5kg:	Yes ☐ No ☐
PCOS:	Yes ☐ No ☐
Infertility:	Yes ☐ No ☐
Miscarriages (number):	
Current Medication:	
Lipid modifying agent:	Yes ☐ No ☐
Anti-hypertensives:	Yes ☐ No ☐
Steroids:	Yes ☐ No ☐
Aspirin:	Yes ☐ No ☐
Other medication:	Yes ☐ No ☐
Non Prescription:	Yes ☐ No ☐
Social Status:	
Medical cardholder:	Yes ☐ No ☐
Private Health Insurance:	Yes ☐ No ☐
Occupation:	
Educational attainment:(SLAN) 1. ☐ Some Primary 2. ☐ Primary only 3. ☐ Junior Cert 4. ☐ Leaving Cert	 5. ☐ Diploma / Cert 6. ☐ Primary degree 7. ☐ Post G / Higher 8. ☐ Refusal
Income (Euro per/yr): 2010: A. <10,0000 ☐ B. 10,000 – 19,999 ☐ C. 20,000 – 29,999 ☐	 D. 30,000 – 39,999 ☐ E. 40,000 – 49,000 ☐ F. 50,000 + ☐
Smoker: Past Current Never	☐ ☐ ☐
Alcohol unit/week:	

Patient name:_____
DOB:_____
Address_____
_____
_____

BN:_____

Index Pregnancy History:	
Parity:	
Gravidy:	
Booking weight (kg):	
Booking BMI (kg/m²):	
Booking BP:	
Weight gain pregnancy (kg):	
Onset of Pregnancy induced HTN:	Yes ☐ No ☐
Onset of preeclampsia:	Yes ☐ No ☐
Polyhydramnios:	Yes ☐ No ☐
Week of GDM diagnosed:	
HbA1c 1st available measure:	
OGTT result at diagnosis GDM (mmol/L):	
Insulin use:	Yes ☐ No ☐
No. subsequent pregnancy with GDM:	
No. subsequent pregnancies:	
Neonatal Outcome:	
Stillbirth:	Yes ☐ No ☐
Neonatal Death:	Yes ☐ No ☐
Congenital malformation (EUROCAT):	
NNU admission:	Yes ☐ No ☐ Number days:
Mode of Delivery: • SVD • LSCS • Instrumental	 Yes ☐ Yes ☐ Yes ☐
Gestational size at delivery Kg:	
Shoulder dystocia:	Yes ☐ No ☐
At discharge: • Breastfeeding after birth Continued Breastfeeding at home: • Breast feeding and Bottle • Bottle only	 Yes ☐ No ☐ Yes ☐ No ☐ months: ☐ Yes ☐ No ☐ Yes ☐ No ☐