Gestational Diabetes Mellitus - Diagnostic Implications During Pregnancy and Follow-Up

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Diagnostic Implications During Pregnancy and Follow-Up

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LUND
UNIVERSITY

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Abstract

The overall objective of the present thesis was to assess diagnostic implications for diagnosis of gestational diabetes (GDM) during pregnancy (Papers I and V) and for diagnosis of type-2 diabetes at follow-up after pregnancy (Papers II and IV), and the relative contributions of maternal body mass index (BMI) and glucose levels in prediction of large-for-gestational-age (LGA) births (Paper III).

Paper I: New diagnostic criteria have been proposed by the International Association of the Diabetes and Pregnancy Study Groups, which will increase the number of women diagnosed with GDM. Using the capillary 2-h glucose concentration from the oral glucose tolerance test (OGTT) as screening criterion for a repeat diagnostic OGTT, we found an increase in the frequency of GDM of 26% compared to the criteria currently used.

Paper II: Thresholds proposed by the World Health Organization for HbA1c had low sensitivity in diagnosis of diabetes and of abnormal glucose tolerance postpartum in the present study cohort. Combined with a fasting glucose test, the performance was no better than when using a fasting glucose test alone. Combination of a fasting glucose test with a lower cut-point of HbA1c may be an alternative approach to select women for an OGTT, in order to identify those who have isolated post-glucose load hyperglycemia.

Paper III: Maternal BMI had a greater impact on the prediction of LGA birth than the 2-h glucose level from the OGTT.

Paper IV: An HbA1c level of ≥ 36 mmol/mol, obtained close to the twenty-eighth week of pregnancy, was associated with a more than fivefold increased risk of diabetes five years after pregnancy. A cut-off level for HbA1c of ≥ 39 mmol/mol, corresponding to the pre-diabetes range outside of pregnancy, could reveal women with postpartum diabetes with high specificity (97%) and high positive predictive value (91%). Due to the low sensitivity, HbA1c does not appear suitable as a screening test to predict diabetes after GDM in all women, but it could be used as a strategy for selecting high-risk women for lifestyle interventions to prevent diabetes, starting already in pregnancy.

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Key words

Gestational diabetes mellitus, oral glucose tolerance test, type-2 diabetes, diagnostic criteria, HbA1c, screening test, follow-up, body mass index, glucose levels, large for gestational age, seasonal variation

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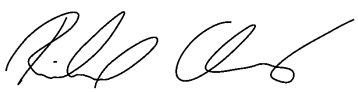
Signature  Date 2017-03-17

Gestational Diabetes Mellitus

Diagnostic Implications During Pregnancy and Follow-Up

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Cover photo by Ulf Lagerholm: "Shoreline just northwest of Vitemölla".

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Alone we can do so little.
Together we can do so much.
Helen Keller, 1880–1968

To my family

Contents

Original papers

This doctoral thesis is based on the following papers, which are referred to in the text by their Roman numerals. The papers are reprinted at the end of the thesis.

I Claesson R, Ekelund M, Berntorp K. The potential impact of new diagnostic criteria on the frequency of gestational diabetes mellitus in Sweden. Acta Obstetricia et Gynecologica Scandinavica. 2013 Oct; 92(10): 1223–6. Epub 2013 Aug 9.

II Claesson R, Ekelund M, Ignell C, Berntorp K. Role of HbA1c in postpartum screening of women with gestational diabetes mellitus. Journal of Clinical and Translational Endocrinology. 2015 Mar; 2(1): 21–5. Open access.

III Berntorp K, Anderberg E, Claesson R, Ignell C, Källén K. The relative importance of maternal body mass index and glucose levels for prediction of large-for-gestational-age births. BMC Pregnancy and Childbirth. 2015 Oct; 15: 280. Open access.

IV Claesson R, Ignell C, Shaat N, Berntorp K. HbA1c as a predictor of diabetes after gestational diabetes mellitus. Primary Care Diabetes. 2017 Feb; 11(1): 46–51. Epub 2016 Sep 28. Open access.

V Katsarou A, Claesson R, Ignell C, Shaat N, Berntorp K. Seasonal pattern in the diagnosis of gestational diabetes mellitus in southern Sweden. Journal of Diabetes Research. 2016 Dec; article ID 8905474, 6 pages. Open access.

Abstract

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Populärvetenskaplig sammanfattning

Under graviditeten riskerar kvinnan att drabbas av ett antal olika graviditetsrelaterade tillstånd, varav graviditetsdiabetes (GDM) är ett av de vanligare. Varken i Sverige eller internationellt har man kunnat enas om gemensamma metoder för att diagnostisera GDM. Flera riskfaktorer har identifierats, bland annat tidigare förekomst av GDM, ärftlighet för diabetes samt övervikt. Man vet också att etniskt ursprung spelar roll. Exempelvis har kvinnor med asiatisk, afrikansk, eller arabisk ursprung en avsevärt högre risk att drabbas än de med nordiskt ursprung. Detta sammanlagt medför att frekvensen GDM skiljer sig mycket åt i olika delar av världen. Andelen som får GDM är i stigande, både i Sverige och internationellt. Detta beror bland annat på den ökande trenden övervikt och fetma i samhället. I södra Sverige diagnostiseras GDM hos cirka 2,6% av alla gravida kvinnor i samband med glukosbelastning, så kallad OGTT (oralt glukostoleranstest). Tidigare definierades GDM som förhöjt blodglukosvärde som upptäcks hos en kvinna när hon är gravid. Världshälsoorganisationen (WHO) delar nu in dessa kvinnor i två grupper: "Diabetes under graviditet" för de kvinnor som har så höga blodsockervärden att de skulle klassats som diabetes om de inte var gravida, och "GDM" för övriga med förhöjda blodsockervärden.

GDM innebär ökade risker för både den gravida kvinnan och det ofödda barnet. Normalt försvinner de förhöjda blodsockervärdena strax efter förlossningen, men risken för framtida diabetesinsjuknande, och därmed associerade komplikationer, är kraftigt förhöjd. Därför bör kvinnor som haft GDM följas upp regelbundet inom vården. Det finns mycket som talar för att även barnet har ökad risk för diabetes, övervikt och hjärt-kärlsjukdom i vuxen ålder. Risken för komplikationer i samband med graviditet och förlossning är också ökad, vilket framför allt beror på en ökad fostertillväxt så att barnet blir större och tyngre än vad det normalt skulle bli. Detta ökar i sin tur risken för förlossningsskador hos kvinnan och att barnet i värsta fall fastnar i förlossningskanalen. Lågt blodsocker hos det nyfödda barnet är också vanligt och att barnet behöver eftervård på avdelning för nyfödda barn (neonatalavdelning) för att det inte mår bra.

En stor multinationell studie, HAPO-studien, visade ett kontinuerligt samband mellan mammans glukosvärden och barnets födelsevikt. Utifrån studieresultaten föreslogs nya diagnostiska gränsvärden för GDM som är betydligt lägre an vad som förekommit i de flesta länder tidigare. WHO antog de nya gränsvärdena 2013, som också är Socialstyrelsens rekommendation sedan 2015. I vår studie, där gravida kvinnor som diagnostiserats med GDM enligt vanlig klinisk praxis genomgick en ny OGTT enligt de nya riktlinjerna, fann vi 26 procents ökning av antalet kvinnor med GDM med de nya diagnoskriterierna.

HbA1c är en analys som ger ett genomsnittligt mått på glukoshalten i blodet de senaste 2–3 månaderna. HbA1c har nyligen godkänts som diagnosmetod för diabetes i den icke-gravida populationen. Att använda HbA1c istället för OGTT vid uppföljningen efter GDM skulle underlätta väsentligt då kvinnan inte behöver vara fastande samtidigt som provtagningen endast tar några få minuter i anspråk. Det skulle dessutom bli billigare för samhället. Våra studieresultat pekar på att HbA1c varken kan användas under eller efter graviditet för att fånga de kvinnor som utvecklar diabetes. Dock verkar HbA1c kunna användas för att selektera fram de kvinnor som har störst risk för att utveckla diabetes, eventuellt kombinerat med ett fasteglukosvärde, och på så sätt minska antalet kvinnor i behov av en diagnostisk OGTT. Förenklad diagnostik skulle förhoppningsvis leda till att fler kvinnor kommer till uppföljningen efter sin graviditet.

Trots god blodglukoskontroll föder kvinnor med GDM i genomsnitt tyngre barn än de som har normala blodglukosvärden. Vi studerade vilket som betydde mest för kvinnans risk att föda ett stort barn, hennes glukosvärde vid OGTT eller hennes kroppsvikt. Det visade sig att kroppsvikten, mätt som body mass index, spelade en större roll än glukosvärdet. Detta belyser vikten av att uppmana kvinnorna till en hälsosam livsstil och att upprätthålla en hälsosam kroppsvikt såväl före som under graviditeten.

Vid genomgång av OGTT-resultaten från alla gravida kvinnor under en treårsperiod fann vi en klar säsongsvariation i antalet kvinnor med GDM-diagnos med en topp under sommaren. De genomsnittliga glukosvärdena följde samma mönster. Fynden kan ha diagnostisk betydelse men orsaken är oklar och måste utredas vidare i kommande studier.

Abbreviations

ACOG	American College of Obstetricians and Gynecologists
ADA	American Diabetes Association
AGA	Adequate for gestational age
ANOVA	Analysis of variance
AUC	Area under the curve
BMI	Body mass index
CI	Confidence interval
CV	Coefficient of variation
EASD	European Association for the Study of Diabetes
EBCOG	European Board and College of Obstetrician and Gynaecology
EDTA	Ethylenediaminetetraacetic acid
FBG	Fasting blood glucose
FPG	Fasting plasma glucose
GDM	Gestational diabetes mellitus
GIGT	Gestational impaired glucose tolerance
GLT	Glucose load test
GWG	Gestational weight gain
IADPSG	International Association of the Diabetes and Pregnancy Study Groups
IDF	International Diabetes Federation
IEC	International Expert Committee
IFCC	International Federation of Clinical Chemistry
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
LGA	Large for gestational age
NDDG	National Diabetes Data Group
NPV	Negative predictive value
NGT	Normal glucose tolerance
NGSP	National Glycohemoglobin Standardization Program
OGTT	Oral glucose tolerance test
OR	Odds ratio
PRS	Perinatal Revision South
PPV	Positive predictive value
RCT	Randomized controlled trial
ROC	Receiver operating characteristic
SD	Standard deviation
SGA	Small for gestational age
WHO	World Health Organization

Background

Classifications of diabetes

The World Health Organization (WHO) has been publishing guidelines for the diagnosis and classification of diabetes since 1965 (1). In the late 1970s, both the WHO and the National Diabetes Data Group (NDDG) produced new diagnostic criteria and a new classification system for diabetes mellitus (2, 3). Since the nomenclature varied and the diagnostic criteria were based on different oral glucose loads, the situation became confused. In 1985, the WHO slightly modified its criteria to coincide more closely with the NDDG values (4). In the late 1990s, new information was available and the classification and the criteria needed to be updated. An American Diabetes Association (ADA) expert group was convened for the purpose, and it published its first recommendations in 1997 (5). The WHO published its update 1999 (6). Generally speaking, the ADA and the WHO groups reached similar conclusions.

The latest updates from the ADA and the WHO are from 2017 and 2006, respectively (7, 8).

It is agreed upon that diabetes, defined by the level of hyperglycemia, can be classified into the following general categories:

1. Type-1 diabetes (due to autoimmune β-cell destruction, usually leading to absolute insulin deficiency).
2. Type-2 diabetes (due to a progressive loss of β-cell insulin secretion, frequently with a background of insulin resistance).
3. Gestational diabetes mellitus (GDM), which is described below.
4. Specific types of diabetes due to other causes, e.g. monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced diabetes (such as with glucocorticoid use, in the treatment of HIV/AIDS, or after organ transplantation).

This thesis focuses on diagnostic implications for and follow-up of GDM, which also covers the diagnostics of type-2 diabetes.

Gestational diabetes mellitus

Definition

GDM was first defined by O'Sullivan in 1961, as "carbohydrate intolerance of varying severity with onset or first recognition during pregnancy"(9).

In 2013, the WHO introduced the term "hyperglycemia first detected at any time during pregnancy", with the following categories (10):

- Diabetes mellitus in pregnancy, diagnosed by the 2006 WHO criteria for diabetes in non-pregnant women
- Gestational diabetes mellitus: hyperglycemia below the thresholds for diabetes outside of pregnancy, but with the risk of adverse pregnancy outcomes.

The most recent definition comes from the ADA (2017): "diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation" (7).

The definition of GDM in this thesis is the one stated by the WHO in 1999: "carbohydrate intolerance resulting in hyperglycemia of variable severity with onset or first recognition during pregnancy" (6).

Pathophysiology

The pathophysiology behind the development of GDM is not fully understood, but the maternal changes in metabolism are substantial during pregnancy. The glucose metabolism changes to meet the nutritional demands of the mother and fetus (11, 12). As early as at the end of the first trimester, significant progressive alterations in all aspects of glucose metabolism occur in women with normal glucose tolerance (13). From the second trimester onwards, pregnant women become increasingly insulin resistant (14). In the third trimester, maternal fasting insulin levels increase by over 30%, while fasting glucose concentrations decrease by about 10%, despite increased insulin resistance, mainly due to increased plasma volume, increased use of glucose, and inadequate production of glucose (12, 13, 15). To maintain glucose homeostasis, a concomitant compensation in insulin production is required by the β-cells, but in women with GDM, -cell function is decreased by 30% to 70% relative to that in women who maintain normal glucose tolerance during pregnancy (12). On average, women with GDM have higher fasting glucose concentrations. Basal hepatic production, however, is not different from that in women without GDM (13).

Significant alterations also occur in lipid metabolism, and circulating lipids and amino acids are also important nutrients for the fetus (15, 16). Several hormones and cytokines are elevated in the maternal circulation during pregnancy, leading to metabolic effects. Potential hormones include human placental lactogen, placentally derived human growth hormone, progesterone, prolactin, leptin, and cortisol (12, 17). In addition, tumor necrosis factor-α is secreted by the placenta and cytokines are secreted from adipose tissue, all of which contribute to postprandial insulin resistance, mainly in peripheral tissues (11, 12, 18). Finally, the potential role of other factors, such as free fatty acids and adipocytes, may also contribute to the insulin resistance of pregnancy (12). There is usually an immediate decrease in insulin resistance after delivery, illustrating the role of placental factors.

A recent large study characterised the effects of pregnancy on maternal metabolism across a wide range of metabolic and inflammatory measures. The metabolic effects of pregnancy were shown to be exceptionally large, gradually increasing across the trimesters, and generally normalizing within 3–6 months postpartum (18).

There are pathophysiological similarities between GDM and type-2 diabetes, and GDM can therefore be regarded as an early stage in the development of type-2 diabetes (19). Genetic links between GDM and type-2 diabetes have been confirmed (11, 20, 21). Furthermore, metabolomics studies have suggested that the metabolic signatures of hyperglycemia in type-2 diabetes and GDM are, in part, similar, while epigenetic studies and—most recently—studies of the gut microbiome are continuously evolving (11, 22, 23).

Risk factors and adverse outcomes associated with GDM

Any woman can develop hyperglycemia during pregnancy, but some women are at greater risk. GDM is strongly associated with being overweight or obese, and the risk of developing GDM is doubled for pregnant women who are overweight (body mass index (BMI) 25.0–29.9 kg/m^2); this risk increases to fourfold for pregnant women who are obese (BMI ≥ 30.0 kg/m^2) (24). Other significant risk factors for developing GDM are having a family history of type-2 diabetes, previous GDM, unexplained intrauterine fetal death, ethnicity (Mediterranean, South Asian, African black, North African, Caribbean, Middle Eastern, hispanic) and a previous macrosomia, usually 4,000 g or 4,500 g regardless of the fetal gestational age (25). These are also risk factors for type-2 diabetes, and they can be used as indicators for screening in early gestation, with the primary aim of detecting pre-gestational diabetes—as recently proposed by the European Board and College of Obstetrics and Gynaecology (EBCOG) (25).

Other risk factors for GDM include being a woman of older maternal age, where the risk increases with advancing age. The risk for a woman over 40 years old is

approximately five times as high as for a woman under 20 years of age (26, 27). Abnormal intrauterine growth of female fetuses correlates with their future risk of developing GDM. Based on Swedish material, being born either small for gestational age (SGA) or large for gestational age (LGA) has been shown to double that risk (28). In a systemic review and meta-analysis, women with polycystic ovary syndrome were shown to have a significantly higher risk of developing GDM (odds ratio (OR) = 3.4) (29).

Obesity is a strong, potentially modifiable risk factor for GDM. A reduction in risk has been reported in relation to physical activity before and during pregnancy (26). On the other hand, the Vitamin D And Lifestyle Intervention for GDM prevention (DALI), recently showed that if they combined healthy eating and physical activity—as opposed to one of them alone—women achieved substantially less gestational weight gain (GWG) than controls by 35–37 weeks (OR = –2.02). Despite this reduction, there were no improvements in fasting or post-load glucose or insulin concentrations. Birth weight, LGA rates and SGA rates were similar (30).

In a recently published meta-analysis, the pooled GDM recurrence rate was 48%. A significant association between ethnicity and GDM recurrence rate was found. Non-hispanic white women had a lower recurrence rate then women of other ethnicities, (39% and 56%, respectively). Primiparous women had a lower recurrence rate than multiparous women, (40% and 73%, respectively) (31). Parity is a variable that interacts with other risk factors, but after adjustment it has been shown to be associated with an increased risk of diabetes after the fourth delivery (32).

A family history of type-2 diabetes increases the risk of GDM. A systematic review reported ORs of 1.6 to 3.0 (26). Ethnicity has been proven to be an independent risk factor for GDM, which varies in prevalence in direct proportion to the prevalence of type-2 diabetes in a given population or ethnic group. The prevalence is higher in non-European populations (33-35). This may be partly explained by differences in insulin secretion and action. For the South Asian population, both lower thresholds in relation to the risk of type-2 diabetes and an ethnic difference in leptin concentration have been suggested (36, 37). Soluble leptin receptor, a potential marker of leptin resistance, has been found to be inversely associated with the risk of type-2 diabetes, independently of leptin concentrations. A recent publication from Sommer et al. showed that there was an independent inverse association between soluble leptin receptor and GDM, with the lowest risk of GDM being observed with higher soluble leptin receptor concentrations. However, in contrast to earlier findings, the soluble leptin receptor levels did not differ significantly in different ethnic groups and did not explain ethnic differences in GDM risk (38).

Alternations in insulin resistance and secretion are pivotal in the pathophysiology of GDM. However, these factors influence the homeostasis of many metabolites besides glucose. In GDM, especially during the third trimester, there is an associated increase in triglycerides and a decrease in high-density lipoprotein concentration (17). Women

with a history of GDM are more likely to have hypertension, vascular dysfunction, impaired endothelium-dependent vasodilatation, and higher carotid artery intima-media thickness (16). These signs of cardiovascular disease are not fully explained by the higher BMI typical of women with a history of GDM (16). As a consequence, there is a threefold increased risk of subsequent metabolic syndrome postpartum (39).

GDM, high pre-pregnancy BMI, and GWG are independently associated with an increased risk of adverse perinatal outcomes, including macrosomia, operative delivery, and shoulder dystocia (40). In GDM, such complications have a continuous relationship with maternal glucose concentrations during the oral glucose tolerance test (OGTT) (41). The intrauterine excess of nutrients and the enhanced insulin production that results from it both contribute to fetal growth (42). In a systematic review from 2012, Wendland et al. described risks of GDM according to the WHO criteria from 1999 and the International Association of Diabetes in Pregnancy Study Groups (IADPSG) criteria. Significant risk ratios using the respective criteria were 2.2 and 1.4 for macrosomia, 1.4 and 1.2 for caesarean delivery, and 1.7 (both criteria) for LGA and pre-eclampsia (43).

Fetuses that are exposed to maternal diabetes have a higher risk of abnormal glucose homeostasis in later life beyond that attributable to genetic factors (28, 44-46). Indeed, it is currently widely accepted that an abnormal in utero stimulus or 'insult' has the ability to disrupt the normal pattern of fetal development, permanently changing its body's structure, physiology and metabolism, thereby predisposing to chronic diseases in later life. This phenomenon is referred to as fetal or gestational programming (46-48). This hypothesis was first introduced by David J. Barker (47), who proposed "that poor fetal and early post-natal nutrition imposes mechanisms of nutritional thrift upon the growing individual", leading to increased rates of future cardiovascular disease (49), hypertension (50), and type-2 diabetes (47, 49). The role of intrauterine hyperglycemia in programming of the fetus was, however, recently questioned by Donovan and Cundy, who suggested that parental obesity as a confounder has not been taken into account (51).

Screening and diagnosis

Screening recommendations range from the inclusion of all pregnant women (universal) to the exclusion of all women except those at risk (selective). Over the years, there has been controversy regarding screening tests, diagnostic tests, and the level of hyperglycemia that is diagnostic of GDM, and still to date there is no international or Swedish consensus regarding which criteria should be used (52, 53). Lindqvist et al. found that four different regimes were used in Sweden in 2011–2012: universal screening with a 2-hour cut-off value of 10.0 mmol/l; selective screening with a 2-hour cut-off value of 8.9 mmol/l; selective screening with a 2-hour cut-off value of 10.0 mmol/l; and selective screening with a 2-hour cut-off value of 12.2

mmol/l (53). Random glucose measurements and risk factor-based screening have a sensitivity to detect GDM of about 50% each (53-56), and approximately 70% when combined, which is the method mostly used in Sweden outside of our area (54).

The basis for the diagnosis of GDM was laid down by O'Sullivan and Mahan in the 1960s (57). After investigating the distribution of plasma glucose values of pregnant women, these authors proposed diagnostic criteria for GDM based on a 3-h 100-g OGTT. Evidence for adverse perinatal outcome was not found until later (41, 58, 59). These criteria were widely used, especially in the USA.

For the last three decades, different groups and organizations have proposed guidelines with diagnostic criteria for the diagnosis of hyperglycemia during pregnancy, based on the latest evidence or best knowledge. The most commonly used criteria are listed in Table 1.

Table 1.
The most commonly used glucose criteria for the diagnosis of gestational diabetes mellitus

		Lower limits of venous plasma glucose (mmol/l)				
Organization, year	Tolerance test used	Fasting	1 h	2 h	3 h	Diagnosis
EASD, 1991 (60)	Fasting, 75-g OGTT	7.0	11.0	9.0	NA	≥ 1 positive
WHO, 1999 (6)	Fasting, 75-g OGTT	7.0	NA	7.8	NA	≥ 1 positive
IADPSG, 2010 (61)	Fasting, 75-g OGTT	5.1	10.0	8.5	NA	≥ 1 positive
WHO, 2013 (10) ADA, 2017 (7) †	Fasting, 75-g OGTT	5.1–6.9*	10.0	8.5–11.0*	NA	≥ 1 positive
ACOG, 2013 (62)†† ADA, 2017 (7) †	Non-fasting, 50-g GLT	NA	7.8**	NA	NA	
Carpenter/Coustan	Fasting, 100-g OGTT	5.3	10.0	8.6	7.8	≥ 2 positive
NDDG	Fasting, 100-g OGTT	5.8	10.6	9.2	8.0	≥ 2 positive

†ADA recommends either one-step or two-step screening, as described in the text.
††ACOG recommends a two-step screening.
*If above the upper limit, it is classified as diabetes in pregnancy.
**7.5 or 7.2 mmol/l in high-risk ethnic populations.
ADA, American Diabetes Association; ACOG, American College of Obstetricians and Gynecologists; EASD, European Association for the Study of Diabetes; GLT, glucose load test; IADPSG, International Association of the Diabetes and Pregnancy Study Groups; NA, not applicable; NDDG, National Diabetes Data Group; OGTT, oral glucose tolerance test; WHO, World Health Organization.

While there is a clear relationship between increased plasma glucose levels during pregnancy and adverse fetal and maternal outcomes, there are some data to suggest that current diagnostic criteria for GDM are too restrictive and that lesser degrees of hyperglycemia also increase the risk (59, 63, 64). The extent to which adverse outcomes associated with GDM may be explained by confounders (including obesity, advanced maternal age, and associated medical complications) is unclear (10, 65). Various cohort studies have addressed this question, using different GDM diagnostic procedures and criteria (41, 64, 66). The most comprehensive study was the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study, an international

multicenter cohort of 25,505 pregnant women tested with a 2-h 75-g OGTT and then followed through pregnancy to detect primary and secondary outcomes (41). After adjustment for multiple potential confounders, the study showed linear associations between plasma glucose levels and adverse neonatal outcomes, and that these associations were independent of other known risk factors for these outcomes.

Until 2015, there had been no uniform national guideline for screening and diagnosis of GDM in Sweden. The Swedish Board of Health and Welfare has now taken action on this issue and has adopted the new WHO and IADPSG thresholds for the diagnosis of GDM, but leaves it up to the local health authorities to specify the strategy for screening (67).

Screening program for GDM in southern Sweden

In the early 1990s in the counties of Skåne and Blekinge in southern Sweden, the screening procedure was still based on random glucose measurements. Åberg et al. found that infants previously born of women who were subsequently diagnosed with GDM in a later pregnancy were heavier than in the control group. They concluded that GDM might have been undetected in previous pregnancies (68). Thus, from 1991 onward, a change in the screening program was introduced and it was implemented in the whole region of Skåne and Blekinge from 1995. Since then, screening of GDM with OGTT has been offered to all women in the twenty-eighth week of gestation, and also in gestational week 12 if there has been a history of GDM in previous pregnancies or if there is a first-degree relative with diabetes. A simplified OGTT is used, omitting the initial fasting glucose measurement. This screening procedure was used unchanged during the recruitment period for the studies included in this thesis. The program has previously been shown to include more than 93% of the eligible women, with 2% of the women not being able to perform the OGTT and less than 3% of the women refusing (55).

A 75-g OGTT is performed after overnight fasting at the local antenatal clinic. The HemoCue blood glucose system (HemoCue AB, Ängelholm, Sweden) is used for immediate analysis of capillary glucose concentrations. To ascertain the quality of the individual testing, double sampling is used, with acceptance of a divergence of ≤ 0.3 mmol/l. The highest test result is regarded as the diagnostic value (55). If the degree of divergence is not reached, a third sample is taken, and if the divergence between two of the samples is still not acceptable, the equipment is checked and the OGTT is not regarded as being valid.

The diagnostic criteria for GDM used in clinical practice are a slight modification of those recommended by the EASD, defining GDM as a 2-h capillary blood glucose concentration of ≥ 9.0 mmol/l (60, 69). In 2004, routine glucose measurements in Sweden switched from blood glucose measurements to plasma glucose measurements, and a transformation factor of 1.11 was agreed on (70), resulting in a 2-h threshold value of 10.0 mmol/l for capillary plasma glucose to define GDM. According to

clinical routines, women with blood glucose concentrations of 7.8–8.9 mmol/l (plasma glucose 8.9–9.9 mmol/l) are offered a second OGTT within a week, and if the glucose levels are still in the intermediate range the woman is referred to a dietician for advice. Otherwise, no further action is taken (55).

Women diagnosed with GDM are referred to specialist antenatal care for intensified maternal and fetal surveillance. These women are given advice on diet and physical activity, and are closely monitored using self-tests for blood glucose. If the treatment goals for glucose levels are not achieved, treatment with insulin is started. The intensified fetal surveillance involves more frequent checks by midwives and obstetricians, such as extended ultrasound examinations and cardiotocography.

While the ADA has always recommended that venous plasma should be used for diagnostic purposes (71), WHO provided cut-off limits for both venous and capillary glucose concentrations in the guidelines from 1999 (6). However, in the updated guidelines from 2006, only the use of venous sampling for glucose measurements is recommended (8). Nevertheless, capillary sampling is still commonly used in Sweden for diagnostic purposes, both during and outside of pregnancy.

Prevalence

GDM

The prevalence of GDM in a population reflects the prevalence of type-2 diabetes within that population. In population-based studies, prevalence generally ranges from 2% to 6%, sometimes with much higher values (10−22%) in certain populations (26). But there have also been studies from northern Europe that have found a prevalence of less than 1% (72). Observed differences may very well be explained by differences in predisposing risk factors (26). The frequency of GDM is also influenced by the definition used and by the screening activity for GDM, which makes it difficult to compare prevalence rates between populations (56, 73).

There is a general trend of increase in prevalence of GDM worldwide (73, 74). In southern Sweden, the prevalence of GDM increased from 1.9% in 2003 to 2.6% in 2012 (75). From a population-based study using the Swedish national medical birth registry data, it was recently reported that all types of diabetes in pregnancy increased over a 15-year time period (1998–2012). Mothers' pre-pregnancy BMI was the key factor explaining the increase in GDM/type-2 diabetes.(76).

Another factor that could influence the prevalence of GDM is the time point of the year when the OGTT is performed. Seasonality in the onset of type-1 diabetes is well documented (77), but less is known about seasonality in the diagnosis of type-2 diabetes and GDM. Many factors vary with season, including the nutritional quality of foods, temperature, the number of hours of sunshine, and vitamin D synthesis.

Maternal vitamin D deficiency in early pregnancy has been associated with increased risk of GDM (78). Doró et al. reported an increased incidence of type-2 diabetes onset in winter (79), but in contrast, Schmidt et al. reported a fourfold increase in the frequency of GDM in the summer compared to winter, which they related to increased 2-h glucose levels in the OGTT at higher ambient temperatures (80). Seasonality of GDM was also reported in two recent studies from Australia (81, 82), whereas two previous studies found no clinically significant evidence of any seasonal variation in the prevalence of GDM or in 2-h glucose levels in the OGTT (83, 84).

Type-2 diabetes

During the twentieth century, and to date, the prevalence of type-2 diabetes has increased dramatically, and it is now considered to be one of the main threats to human health (85). Already in 1921, Dr Elliot Joslin reported a doubling of diabetes in three decades (86). The WHO estimated in 1998 that there would be 150 million people aged 20 years or more living with diabetes in 2000, and by 2025 this would have risen to 300 million (87). However, in 2016 the WHO released its Global Report on Diabetes, where the number of people affected by diabetes was revised (88). The new estimate was that 422 million adults were living with diabetes in 2014 globally, roughly 90 per cent of whom have type-2 diabetes, and the new prediction is that by 2040 we will have about 642 million people with diabetes. The highest increase in the prevalence (by per cent) are predicted to be in Africa (+140%), Southeast Asia (+80%), and in South and Central America (+65%), while the increase in Europe is estimated to be lower (approximately 40%) (88). The global age-standardized prevalence of diabetes has almost doubled since 1980, rising from 4.7% to 8.5% in the adult population, which reflects an increase in associated risk factors such as being overweight or obese (88).

The prevalence of known type-2 diabetes in Sweden has varied between 4% and 6% in different studies (89-93). The various results may partly be explained by differences in diagnostic methods, but they may also relate to demographic differences in the populations under study.

For decades, the diagnosis of diabetes was based on glucose criteria, either the fasting plasma glucose (FPG) or the 2-h value in the 75-g OGTT (94). In 2009, an International Expert Committee that included representatives of the ADA, the International Diabetes Federation (IDF), and the European Association for the Study of Diabetes (EASD) recommended the use of the HbA1c test to diagnose diabetes (95), which was adopted by the ADA in 2010 (94) and by the WHO in 2011 (96). A diagnostic cut-off point of ≥ 6.5% (≥ 48 mmol/mol) was recommended, based on the risk of developing microvascular complications such as retinopathy. No formal recommendations on the interpretation of HbA1c levels below this cut-off point were made by the WHO (96). However, the IEC recommended that high-risk individuals with HbA1c levels between 6.0% (42 mmol/mol) and 6.4% (47 mmol/mol) should

be considered for diabetes prevention and interventions (95). The ADA suggested that HbA1c levels between 5.7% (39 mmol/mol) and 6.4% (47 mmol/mol) indicate intermediate hyperglycemia (94).

The current criteria for the diagnosis of type-2 diabetes are listed in Table 2.

Table 2.
Values used for the diagnosis of hyperglycemic conditions, according to the WHO (6, 96) and the ADA (7)

Glucose tolerance	HbA1c (mmol/mol)	Venous P-glucose (mmol/l)		Capillary P-glucose (mmol/l)	
		Fasting	2-h PG (OGTT)	Fasting	2-h PG (OGTT)
IFG according to:					
ADA		5.6–6.9	< 7.8		
WHO		6.1–6.9	< 7.8	6.1–6.9	< 8.9
IGT according to:					
ADA		5.6–6.9	7.8–11.0		
WHO		6.1–6.9	7.8–11.0	6.1–6.9	8.9–12.1
Prediabetes according to:					
ADA	39–47				
WHO	42–47				
Diabetes according to:					
ADA and WHO	≥ 48	≥ 7.0	≥ 11.1	≥ 7.0	≥ 12.2

ADA, American Diabetes Association; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; P, plasma; PG, plasma glucose; WHO, World Health Organization.

HbA1c can be used for diagnosis of pre-diabetes (impaired fasting glucose [IFG] and/or/impaired glucose tolerance [IGT]), but it is not possible to tell what kind of pre-diabetes it is.

The diagnosis of diabetes must be confirmed with a repeat test using the same method, unless the value of HbA1c and any of the glucose criteria are both above the diagnostic thresholds.

Benefits of treatment

It has previously been demonstrated that the development of type-2 diabetes can be reduced by 58% over a 4-year period by lifestyle interventions in women with a history of GDM (97). The effect was sustained during a 10-year follow-up period (98). However, whether GDM can be prevented through antenatal lifestyle interventions—even with limitation in excess gestational weight gain—is disputed (99). Randomized controlled trials (RCTs) have provided variable evidence that lifestyle interventions are effective in the prevention of GDM (100). The most recent

study, the DALI Lifestyle Study, a large multicenter RCT conducted in nine European countries in 2012–2015, concluded that there were no significant differences between groups in the development of GDM and SGA or LGA babies (30).

Two RCTs have shown that treatment of mild GDM is effective in reducing macrosomia, LGA, shoulder dystocia, and pre-eclampsia/hypertensive disorders in pregnancy. The risk reduction for these outcomes is generally large, the number need to treat is low, and the quality of evidence is adequate to justify treatment of GDM (10, 101, 102).

Follow-up postpartum

Women with GDM remain a high-risk group for the development of IFG, IGT, type-2 diabetes, and cardiovascular disease, including the metabolic syndrome postpartum (103-105). A cumulative diabetes incidence of 30–50% within 5–10 years after GDM has been described (106-108). Women with GDM have a 7.7-fold increased risk of future development of type-2 diabetes (104). Follow-up after GDM is of utmost importance to promote a healthy lifestyle and to identify women who are in need of more intense preventive measures or treatment for postpartum diabetes (103, 105). However, studies have repeatedly shown poor compliance with recommended guidelines in clinical practice, and the women fail to attend the postpartum visit, even in a research setting (109-112). A major challenge in public healthcare is to identify individuals who have the highest risk and to motivate them to come to the follow-up after the delivery (103, 113). Easy, cost-effective, and less time-consuming screening strategies are required to capture as many women as possible who are at risk of developing type-2 diabetes. In this context, the HbA1c test appears to be attractive and its validity as a screening tool for abnormal glucose metabolism after GDM has only been examined in a few studies, with somewhat conflicting results (111, 114-118).

Since recurrence rates for GDM are high (30–84%), women with previous GDM who are planning future pregnancies should be informed appropriately before their next pregnancy (31, 119).

Factors affecting the use of HbA1c as a diagnostic test

Conditions that affect non-enzymatic glycation of hemoglobin (120) or red blood cell survival time (121), such as hemolytic anemia and anemia of chronic disease, will lower the HbA1c level, which could in turn lead to false negative result.

Another major factor that influences HbA1c levels is iron deficiency, which may prolong red cell survival and increase HbA1c levels (122). During pregnancy, iron deficiency is more common, just as anemia and iron replacement, which all affects the levels of HbA1c and does the interpretation of HbA1c hazardous (123).

In early pregnancy, the HbA1c levels fall in most women—which is thought to be related to increased red cell production and a decrease in fasting blood glucose levels—and reach a nadir in the early second trimester (124), when levels are consistently reported to be lower than in non-pregnant controls (125). In later pregnancy, the reported HbA1c levels have varied. This could be explained by differences in iron status between groups and in the methods used for the diagnosis of GDM (125). In addition, it has been demonstrated that there is increased turnover of red blood cells in late pregnancy (126).

In certain ethnic communities, e.g. African and Mediterranean, hemoglobinopathies, congenital variants of the hemoglobin molecule, are more common—usually with a lower HbA1c as a result. If this is suspected, there are some recommendations not to use HbA1c for diagnosis, but to use glucose-based criteria instead (127).

Even without hemoglobinopathies, there are reports of different HbA1c ranges between some ethnic groups. In a recent study of women with previous GDM, by Waage et al., western European women have lower values than women from ethnic minorities (128). Similar results have been shown from the USA in the non-pregnant population, where African Americans have higher HbA1c values than Mexicans and non-hispanic whites (129). The mechanism behind the ethnic variations is not known. Differences in the prevalence of conditions affecting erythrocyte turnover, genetic glycation differences, and differences in glycemia that are not represented by the fasting and post-load glucose levels, are all possible factors (125).

Aims of the investigations

The specific aims of the individual studies are given below.

I To determine how the IADPSG and WHO 1999 criteria would affect the number of women diagnosed with GDM, compared to current guidelines.

II To compare the performance of HbA1c with established glucose criteria during an OGTT and to assess HbA1c as a screening test for undiagnosed diabetes and pre-diabetes after GDM.

III To evaluate the relative importance of maternal BMI and glucose levels in prediction of LGA births.

IV To investigate third-trimester HbA1c as a predictor of diabetes after GDM.

V To examine seasonal patterns in glucose tolerance and in the diagnosis of GDM.

Subjects and study design

I, II. The Malmö study

Subjects

Until recently, all women diagnosed with GDM in the region of Malmö and Trelleborg in southern Sweden have been referred to the Department of Endocrinology in Malmö for follow-up during pregnancy. Women referred between 1996 and 1999 were invited to take part in a 5-year follow-up study, including measurement of HbA1c and a repeat 75-g OGTT after overnight fasting as soon as possible after referral (median 9 days, interquartile range 6 days) (107). Venous samples were drawn at 0, 60, and 120 min, and immediately analyzed in a HemoCue blood glucose meter for determination of glucose concentration. The follow-up included a repeat OGTT and an HbA1c test at 1, 2, and 5 years after delivery. Out of 188 consecutive patients, 182 agreed to be enrolled. The study design has been described previously (107). The study protocol was approved by the ethics committee of Lund University (LU 112–96).

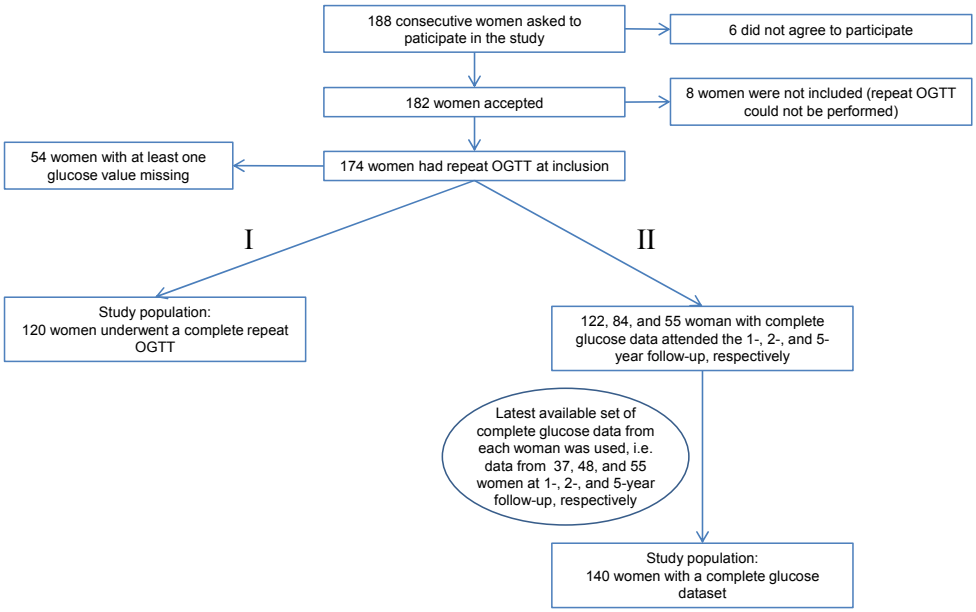

Figure 1.
Flow chart of the study populations in Paper I and II.
OGTT, oral glucose tolerance test.

Study design, Paper I

Altogether, 182 women took part in the follow-up study. Eight of them were not included in the analysis because a repeat OGTT could not be performed at the start of the study. Of the 174 women who remained, at least one glucose value was missing during the OGTT in 54 of them. Hence, 120 women underwent a complete repeat OGTT.

Women in whom the GDM diagnosis was consistent with the modified EASD criteria were identified and the additional number of women identified when applying the IADPSG criteria to this group was determined. Similarly, the number of women identified as having GDM using the WHO 1999 criteria was calculated.

The diagnostic threshold values prescribed by the different criteria are presented in Table 3.

Table 3.
Threshold values for the diagnosis of GDM according to the different criteria

Criteria	Venous plasma glucose concentration threshold (mmol/l)[a]		
	Fasting	1-h	2-h
Modified EASD	NA	NA	≥ 8.5
IADPSG	≥ 5.1	≥ 10.0	≥ 8.5
WHO	≥ 7.0	NA	≥ 7.8

[a]One or more of these values must be equalled or exceeded for the diagnosis of GDM.
GDM, gestational diabetes mellitus; EASD, European Association for the Study of Diabetes; IADPSG, International Association of the Diabetes and Pregnancy Study Groups; WHO, World Health Organization; NA, not applicable.

Study design, Paper II

This study was based on the same cohort as in Paper I. Of the 182 eligible women, a total of 174 women were finally included. Only women with complete glucose data at follow-up, i.e. simultaneous measurements of fasting and 2-h glucose values during the OGTT, in addition to an HbA1c test, were selected for the evaluation. Altogether, 122, 84, and 55 women attended the 1-, 2-, and 5-year follow-up, respectively. To ensure the longest possible follow-up time, the latest available set of complete glucose data from each woman was used, i.e. data from 37, 48, and 55 women at 1-, 2-, and 5-year follow-up, respectively. A standard 75-g OGTT was performed after overnight fasting. Venous blood samples were drawn in duplicate at 0 and 120 min for determination of glucose concentrations, and the mean value was calculated. A blood sample for determination of HbA1c was collected in a tube containing EDTA. Weight and height were recorded and BMI was calculated.

Based on the results of the OGTTs, four subgroups were defined according to the WHO 1999 criteria based on glucose measurement in whole blood:

- Normal glucose tolerance (NGT), fasting blood glucose (FBG) < 5.6 mmol/l and 2-h blood glucose (2-h BG) < 6.7 mmol/l
- Impaired fasting glucose (IFG), FBG 5.6–6.0 mmol/l and 2-h BG < 6.7 mmol/l
- Impaired glucose tolerance (IGT), FBG < 6.1 mmol/l and 2-h BG 6.7–9.9 mmol/l; and
- Diabetes mellitus, FBG ≥ 6.1 mmol/l and/or 2-h BG ≥ 10 mmol/l (6).

Glucose homeostasis was also determined based on HbA1c levels according to the WHO and ADA recommendations: ≥ 48 mmol/mol (≥ 6.5%) suggesting diabetes; 39–47 mmol/mol (5.7–6.4%) suggesting high risk (pre-diabetes); and < 39 mmol/mol (< 5.7%) suggesting normal glucose homeostasis (96, 130). For comparison, the combined category "IFG and IGT" was used to represent pre-diabetes and the combined category "IFG, IGT, and diabetes" was used to represent

abnormal glucose tolerance. Similarly, HbA1c levels of ≥ 39 mmol/mol (≥ 5.7%) were used to define abnormal glucose homeostasis.

III, IV, V. The Mamma study

Subjects

Recruitment to the prospective Mamma study took place during the years 2003–2005 and involved four of the five delivery departments in the county of Skåne in southern Sweden, covering 86% of all pregnancies in the region (112). Pregnant women representing different glucose categories according to the OGTT were invited to take part in a five-year follow-up study. For the purposes of study IV, only women with GDM according to current clinical criteria (modified EASD) were included. During the recruitment period, OGTT results from the local antenatal clinics were sent to the study coordinator, enabling identification of the test results of women who consented to be enrolled; it also ensured correct sampling technique (55). In total, 11,976 OGTT results were reported. For the purposes of studies III and V, only the first pregnancy was included if a woman had more than one pregnancy during the study period. Likewise, if a woman underwent more than one OGTT during the same pregnancy, only the one performed in pregnancy week 28 was included.

All the women were given verbal and written information about the study in connection with the OGTT at the local antenatal clinic, and they were finally invited to participate by the midwives at the delivery department. The women who accepted the invitation gave their written, informed consent. The study protocol was approved by the ethics committee of Lund University (LU 259–00).

Study design, Paper III

Population-based information was retrieved from the regional perinatal database, Perinatal Revision South (PRS), which was established in 1995 for quality assurance in perinatal care in the southern region of Sweden (131). The PRS is based on approximately 18,000 annual births, and is compiled from data reported by all delivery and neonatal units in the region. The maternal pregnancy characteristics used as exposure variables were maternal age at delivery, parity, BMI, maternal height, and maternal smoking. Information about BMI (kg/m^2) was based on weight and height measured at the first prenatal visit in the first trimester. Gestational age was estimated from expected date of parturition according to ultrasound in the first half of gestation. LGA births, SGA births, and adequate-for-gestational-age (AGA) births

were defined as birth weight greater than +2 standard deviations (SDs), less than −2 SDs and between −2 SDs and +2 SDs of the expected birth weight for gestational age and gender, respectively, according to the Swedish reference curve for fetal growth (132). Of the 11,976 OGTT results, information in the PRS was available for a total of 11,016 pregnancies. When we evaluated the risk factors for LGA, infants with unavailable LGA information were excluded, and this restricted dataset formed the basis of the present evaluation (n = 10,974). The dataset was divided into two parts, with every second woman belonging to the development dataset or the validation dataset.

Study design, Paper IV

A 75-g OGTT was offered to all pregnant women according to the routine procedure in southern Sweden described above. Based on current GDM criteria, 391 women were recruited. HbA1c was measured within two weeks of the diagnosis of GDM. Participants were followed for the development of diabetes by means of an OGTT at 1–2 years and at 5 years after pregnancy—or until the diagnosis of diabetes. Based on the stated country of origin of at least three grandparents, women were grouped according to whether they were of European or non-European origin. Diagnostic criteria during follow-up were those proposed by the WHO (1999) (6). According to the results of the OGTT, women were classified as having NGT, IFG, IGT, or diabetes.

Study design, Paper V

The study was restricted to 11,538 of the 11,976 reported OGTTs after taking the inclusion criteria described above into consideration. Mean monthly temperatures during the study period were obtained from the Swedish Meteorological and Hydrological Institute (http://opendata-download-metobs.smhi.se/explore/?parameter=3#). The OGTT data were used to examine seasonal patterns in glucose tolerance and in the diagnosis of GDM.

The diagnostic criteria for GDM proposed by the WHO in 1999 were used in this paper (6).

Methods

Assays

The HemoCue Glucose system (HemoCue AB, Ängelholm, Sweden) was used for immediate measurement of glucose concentrations (in mmol/l). After the switch to reporting of glucose concentrations in plasma 2004, the HemoCue Glucose 201+ Analyzer was used, converting blood glucose concentrations to equivalent plasma glucose concentrations by using a factor of 1.11 (70, 133).

HbA1c was analyzed by ion-exchange chromatography, Mono S HPLC (134). The within-assay coefficient of variation (CV) (on the Mono S scale) of this method is 0.47–0.94% and the between-assay CV is 1.68%. The Mono S method, together with the reference method from the NGSP (National Glycohemoglobin Standardization Program), is a designated comparison method in the International Federation of Clinical Chemistry (IFCC) Reference System (135). Numbers given in Mono S % can be converted to NGSP units (%) and IFCC units (mmol/mol) using the regression equations developed by the IFCC Working Group (135).

Statistical analysis

Paper I

The study was designed to determine how the new IADPSG criteria would affect the number of women diagnosed with GDM in southern Sweden compared to present guidelines, and to evaluate how the WHO 1999 criteria would affect these results. No specific statistical analysis was needed.

Paper II

The agreement between diagnoses resulting from HbA1c and OGTT criteria was estimated by constructing cross-tables. The coefficient ( ) was calculated, where the closer the value is to 1, the better the agreement (136). Spearman's correlation was used to analyze the relationship between glucose values and HbA1c values. A receiver operating characteristic (ROC) curve was constructed for HbA1c using OGTT as the gold standard for the diagnosis of abnormal glucose tolerance, and the area under the curve (AUC) was calculated. Diagnostic accuracy was assessed using sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Statistical analyses were performed with IBM SPSS Statistics 22 for Windows (IBM Corporation, Armonk, NY). Any *p*-value of less than 0.05 was considered to be statistically significant.

Paper III

Differences in glucose levels between groups were assessed using the Kruskal-Wallis test. Chi-squared tests were performed to test possible differences between the datasets regarding maternal and infant characteristics (i.e. the development dataset and the validation dataset). The correlation between maternal BMI and 2-h glucose levels was estimated using Pearson's rho correlation, and the linear relationship was estimated using a simple linear regression.

The prediction model for LGA was developed on the development dataset using univariate and multivariable logistic regression analysis. The variables tested were: maternal age (in years; continuous variable), parity 1, parity ≥ 4 (with parity 2–3 as reference), maternal smoking (yes/no), maternal BMI (in kg/m^2; continuous), maternal height (in cm; continuous), and glucose levels (in mmol/l; continuous). Models including class variables or second-degree polynomials were tested, but were abandoned as they performed worse than the models including the linear, continuous variables mentioned. Variables with a crude *p*-value of less than 0.05 in their association with LGA in the univariate model were entered into a multiple model, and variables with a *p*-value of less than 0.05 in the multiple model were entered into the final multiple model. A two-sided *p*-value of less than 0.05 was considered to be statistically significant.

The results obtained from the final multiple model, and two other models for comparison, were applied to the validation dataset. The performance of each model was evaluated by studying the area under the ROC curve (AUC). The variance of each AUC was computed using the method proposed by DeLong et al. (137).

All statistical analyses were performed using Gauss (Gauss™; Aptec Systems Inc., Maple Valley, WA, USA; http://www.aptech.com).

Paper IV

Continuous variables are summarized as means with standard deviations (SDs) or 95% confidence intervals (CIs). Differences between group means were compared using analysis of variance (ANOVA). Logistic regression analysis was used to calculate the ORs and 95% CIs for 5-year diabetes risk in different quartiles of HbA1c levels. A ROC curve was plotted to evaluate the diagnostic performance of HbA1c in diabetes prediction. Sensitivity, specificity, PPV, NPV, and AUC were calculated. Threshold for discrimination was calculated with the Youden index (138).

IBM SPSS Statistics 22.0 for Windows (IBM Corporation) was used for analysis. Two-sided *p*-values of less than 0.05 were considered to be statistically significant.

Paper V

OGTT results from the 3-year study period were grouped together into months and seasons (winter: December–February; spring: March–May; summer: June–August; autumn: September–November). Chi-squared test was used to test for differences in frequencies between months and seasons, and one-way ANOVA was used to test for the corresponding differences in means. Multivariable logistic regression was used to examine whether month or season was associated with the diagnosis of GDM, and multivariable linear regression was used to examine the corresponding associations with 2-h glucose levels. The relationship between mean monthly temperatures and mean monthly 2-h glucose concentrations was evaluated by simple linear regression.

IBM SPSS Statistics 22 for Windows (IBM Corporation) was used for analysis. Two-sided *p*-values of less than 0.05 were considered to be statistically significant.

Results

Paper I. The frequency of gestational diabetes mellitus with different criteria

Based on a 2-h plasma glucose threshold of 8.5 mmol/l, GDM was confirmed in 67% (80/120) of the women (Table 4). Inclusion of the value of the FPG, according to the IADPSG criteria, identified an additional 5.0% (6/120), and inclusion of the 1-h plasma glucose value identified another 13% (15/120). Three of the women identified by the modified EASD criteria had a fasting glucose level of ≥ 7.0 mmol/l, and one of the women who were not identified by these criteria had a fasting glucose level above this threshold.

Table 4.
Frequency of confirmed diagnosis of gestational diabetes mellitus and subjects with glucose values above specific thresholds

Criteria	**GDM diagnosis confirmed n/total (%)**	**GDM diagnosed considering each glucose level sequentially[a] n/total (%)**			**GDM diagnosed considering individual glucose levels[b] n/total (%)**		
		2-h PG	FPG	1-h PG	FPG	1-h PG	2-h PG
Modified EASD	80/120 (67)	80/80 (100)	NA	NA	NA	NA	80/80 (100)
IADPSG	101/120 (84)	80/101 (79)	6/101 (6)	15/101 (15)	47/101 (47)	64/101 (63)	80/101 (79)
WHO	96/120 (80)	96/96 (100)	0/96 (0)	NA	4/96 (4)	NA	96/96 (100)

[a]Additional number of women identified by each threshold starting with the 2-h PG.
[b]Number of women identified by each glucose threshold.
PG, plasma glucose; FPG, fasting plasma glucose; EASD, European Association for the Study of Diabetes; GDM, gestational diabetes mellitus; IADPSG, International Association of the Diabetes and Pregnancy Study Groups; NA, not applicable; WHO, World Health Organization.

Hence, when we applied the IADPSG criteria to the whole study group, 84% (101/120) were diagnosed as having GDM: 80/101 fulfilled the criteria for the 2-h plasma glucose level, 47/101 fulfilled the criteria for the FPG level, and 64/101 fulfilled the criteria for the 1-h plasma glucose level. Accordingly, 79% (80/101) of the women identified by the IADPSG criteria were also identified as having GDM by the modified EASD criteria.

Based on the WHO criteria, 80% (96/120) were diagnosed as having GDM, all on the basis of the 2-h threshold value. Of the 101 women identified by the IADPSG criteria, 93 were also identified by the WHO criteria. Seven of those not identified as having GDM by the WHO criteria had a 1-h plasma glucose concentration of ≥ 10.0 mmol/l and one had a FPG concentration of 5.8 mmol/l. In addition, three of the women who did not meet the IADPSG criteria had 2-h plasma glucose concentrations of between 7.8 and 8.5 mmol/l.

The IADPSG criteria identified 26% (101/80) more women as having GDM than the modified EASD criteria, and the WHO criteria identified 20% (96/80) more women.

Paper II. The performance of HbA1c, for diagnosis and/or screening, during the OGTT at GDM follow-up postpartum

Mean (± SD) values for age and BMI in the women included were 35.4 ± 5.6 years and 26.6 ± 2.3 kg/m^2, respectively. A median (interquartile range) of 26 (21–60) months had elapsed since their GDM pregnancy. Based on the OGTT, 62 women (44.3%) had normal glucose tolerance, 50 (35.7%) had pre-diabetes (13 IFG, 37 IGT), and 28 (20.0%) had diabetes. Of the 37 women with IGT, 12 had FBG values within the IFG range. In eight women, the diagnosis of diabetes was based on the 2-h glucose value alone and in six women it was based on the fasting glucose value alone. In contrast, using the HbA1c criteria for definition, the corresponding figures for normal glucose homeostasis, pre-diabetes, and diabetes were 114 (81.4%), 21 (15.0%), and 5 (3.6%), respectively. In four of the five HbA1c tests that were consistent with a diagnosis of diabetes, the OGTT revealed diabetes, and in the remaining test it revealed IGT. The sensitivity of HbA1c for diabetes diagnosis was 14.3% and the specificity was 99.1%. The agreement between HbA1c and OGTT in classifying diabetes or non-diabetes was poor, as indicated by a coefficient of 0.194.

Altogether, 23 of 140 women (16.4%) met the combined criteria for abnormal glucose tolerance (both OGTT criteria and HbA1c criteria) (Table 5). The consistency in classifying abnormal glucose tolerance between HbA1c and OGTT criteria was 59% (82/140) and was 0.227, indicating poor agreement. Similar results were obtained when evaluating Nordic and non-Nordic women as separate groups (= 0.278 and = 0.166, respectively), or when evaluating the 1-, 2-, and 5-year results separately (= 0.260, = 0.072 and = 0.337, respectively). Combining HbA1c criteria with fasting glucose criteria improved the agreement for the total group to fair

(79%, = 0.596), although it was no better than between FBG criteria alone and OGTT criteria (79%, = 0.599).

Table 5.
Cross-tabulation between HbA1c, fasting blood glucose, and oral glucose tolerance test criteria in categorization of abnormal glucose metabolism

Test criteria	Normal OGTT	Abnormal OGTT
HbA1c ≥ 39 mmol/mol (≥ 5.7%)	3	23
HbA1c < 39 mmol/mol (< 5.7%)	59	55
FBG ≥ 5.6 mmol/l	0	49
FBG < 5.6 mmol/l	62	29
HbA1c ≥ 39 mmol/mol (≥ 5.7%) or FBG ≥ 5.6 mmol/l	3	52
HbA1c < 39 mmol/mol (< 5.7%) and FBG < 5.6 mmol/l	59	26

FBG, fasting blood glucose; OGTT, oral glucose tolerance test.

Correlations between HbA1c and FBG were 0.353 ($p < 0.001$) at 1- to 2-year follow-up and 0.613 ($p < 0.001$) at 5-year follow-up. The corresponding figures for HbA1c against 2-h glucose were 0.380 ($p < 0.001$) and 0.430 ($p < 0.001$), respectively.

A ROC curve was constructed to evaluate the sensitivity and specificity of HbA1c in detection of abnormal glucose tolerance, as defined by the OGTT (Figure 2). The optimal cut-off point of HbA1c for predicting abnormal glucose tolerance was 33 mmol/mol (5.2%) (AUC = 0.708, 95% CI 0.624–0.793), sensitivity was 69.2%, and specificity was 59.7%.

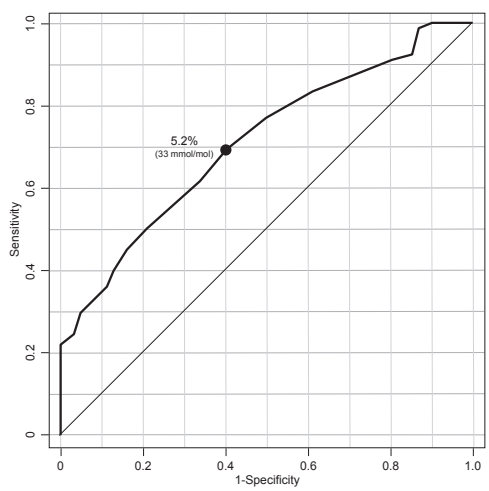

Figure 2.
Receiver operating characteristic curve for HbA1c for detection of abnormal glucose tolerance by the oral glucose tolerance test. The optimal cut-off point for HbA1c is indicated.

Table 6.
Diagnostic indices of various criteria using HbA1c or fasting blood glucose to detect abnormal glucose tolerance

Diagnostic test	n[a]	Sensitivity, %	Specificity, %	PPV, %	NPV, %
HbA1c ≥ 39 mmol/mol (≥ 5.7%)	26	29.5	95.2	88.5	51.8
FBG ≥ 5.6 mmol/l	49	62.8	100.0	100.0	68.1
HbA1c ≥ 39 mmol/mol (≥ 5.7%) or FBG ≥ 5.6 mmol/l	55	66.7	95.2	94.5	69.4
HbA1c ≥ 42 mmol/mol (≥ 6.0%)	17	21.8	100.0	100.0	50.4
HbA1c ≥ 42 mmol/mol (≥ 6.0%) or FBG ≥ 5.6 mmol/l	51	65.4	100.0	100.0	69.7
HbA1c ≥ 31 mmol/mol (≥ 5.0%)	103	83.3	38.7	63.1	64.9
HbA1c ≥ 31 mmol/mol (≥ 5.0%) or FBG ≥ 5.6 mmol/l	108	89.7	38.7	64.8	75.0

[a] Number of women who met cut-off values.
FBG, fasting blood glucose; NPV, negative predictive value; PPV, positive predictive value.

Table 6 shows the sensitivity, specificity, PPV, and NPV of HbA1c and FBG, or a combination of both diagnostic tests, relative to the OGTT (the gold standard) for various cut-offs. Overall, the FBG test alone showed better performance than the HbA1c test alone in detecting abnormal glucose tolerance. Of those who screened positive using the FBG test alone, all had (by definition) abnormal glucose tolerance (13 IFG, 12 IGT, 24 diabetes), as compared to 32% of those who screened negative (25 IGT, 4 diabetes). The combined use of HbA1c and FBG criteria showed performance similar to that with use of the FBG test alone.

We then tested a combination of FBG (≥ 5.6 mmol/l) with various cut-off points of HbA1c to increase the sensitivity and NPV of the combined test. From this, HbA1c ≥ 31 mmol/mol (≥ 5.0%) was judged as an optimal cut-off point, according to which, in addition to the 49 women who screened positive by FBG criteria alone, another 59 women were identified (38 with normal glucose tolerance, 17 with IGT, and four with diabetes by OGTT). Of the remaining 32 women who screened negative using this combination, eight had abnormal glucose tolerance (all IGT) by OGTT.

Paper III. The relative importance of BMI and glucose levels in prediction of LGA births

The frequency of maternal and infant characteristics according to glucose quartile and the corresponding mean 2-h plasma glucose levels are given in Table 7. Of the 2777 women with glucose levels in the upper quartile, 120 (1.1 % of all women) fulfilled the glucose threshold for GDM (2-h plasma glucose concentration ≥ 10.0 mmol/l) and 301 (2.7 % of all women) fulfilled the glucose threshold for gestational impaired glucose tolerance (GIGT), (2-h plasma glucose concentration 8.9–9.9 mmol/l). A linear regression analysis showed a weak, albeit statistically significant, linear association between maternal BMI and glucose levels (increase of 2-h plasma glucose per each BMI-unit: 0.022; 95 % CI 0.017–0.028), with a statistically significant, but weak correlation coefficient (Pearson rho: 0.074; 95 % CI: 0.056–0.093). A ROC curve based on the total dataset revealed that the ability of the 2-h glucose levels to predict LGA births was poor; AUC was 0.54 (95 % CI 0.48–0.60) (Figure 3). Furthermore, there was no apparent natural cut-off point above which there would be an increased risk of LGA in the infant.

Table 7.
Maternal and infant characteristics according to glucose quartiles, and the corresponding 2-h plasma glucose level

Glucose quartiles (mmol/l)	**< 5.7**		**5.7–6.4**		**6.5–7.2**		**> 7.20**		**2-h glucose (mmol/l)**		***p*-value[a]**
	n	%	n	%	n	%	N	%	mean	95% CI	
Total	2,637	23.9	2,783	25.3	2,819	25.6	2,777	25.2			
Maternal age, years											< 0.001
< 20	80	32.5	62	25.2	63	25.6	41	16.7	6.2	6.1–6.4	
20–34	2,148	24.2	2,288	25.8	2,264	25.5	2,180	24.5	6.5	6.4–6.5	
≥ 35	409	21.6	433	22.9	492	26.0	556	29.4	6.6	6.6–6.7	
Parity											0.09
1	128	23.8	134	24.9	141	26.2	135	25.1	6.5	6.4–6.5	
2–3	119	24.1	128	26.0	124	25.2	122	24.7	6.5	6.4–6.5	
≥ 4	16	24.1	15	22.5	15	23.4	20	30.0	6.6	6.5–6.7	
Smoker											< 0.001
No	2,220	23.4	2,408	25.4	2,430	25.6	2,424	25.6	6.5	6.5–6.5	
Yes	341	27.2	309	24.6	333	26.6	271	21.6	6.3	6.3–6.4	
Maternal BMI, kg/m^2											< 0.001
< 18.5	50	25.6	50	25.6	50	25.6	45	23.1	6.4	6.3–6.6	
18.5–24	1,496	25.1	1,569	26.3	1,542	25.9	1,351	22.7	6.4	6.4–6.4	
25.0–29.9	585	22.0	641	24.1	687	25.9	743	28.0	6.6	6.5–6.6	
30–34.9	182	20.8	187	21.4	223	25.5	281	32.2	6.6	6.6–6.7	
≥ 35	83	20.1	103	25.0	93	22.6	133	32.3	6.8	6.7–6.9	
Gestational age, weeks											0.006
< 37	117	20.0	148	25.3	153	26.2	167	28.5	6.7	6.5–6.8	
37–41 + 6	2,345	24.0	2,472	25.3	2,502	25.6	2,452	25.1	6.5	6.4–6.5	
≥ 42 + 0	175	26.5	163	24.7	164	24.8	158	23.9	6.4	6.3–6.5	

Weight for gestational age											< 0.001
SGA	69	23.2	80	26.9	68	22.9	80	26.9	6.5	6.4–6.7	
AGA	2,446	24.2	2,577	25.5	2,578	25.6	2,495	24.7	6.5	6.4–6.5	
LGA	115	20.1	110	19.2	156	27.3	191	33.4	6.7	6.6–6.9	
Gender of infant											0.9
Male	1,407	24.5	1,415	24.5	1,437	25.0	1,479	25.8	6.5	6.4–6.5	
Female	1,228	23.4	1,359	25.8	1,379	26.2	1,292	24.6	6.5	6.5–6.5	

[a]*p*-values obtained by non-parametric tests (Kruskal-Wallis) for difference in glucose level between the specified groups.
AGA, adequate for gestational age; BMI, body mass index; CI, confidence interval; LGA, large for gestational age; SGA, small for gestational age.

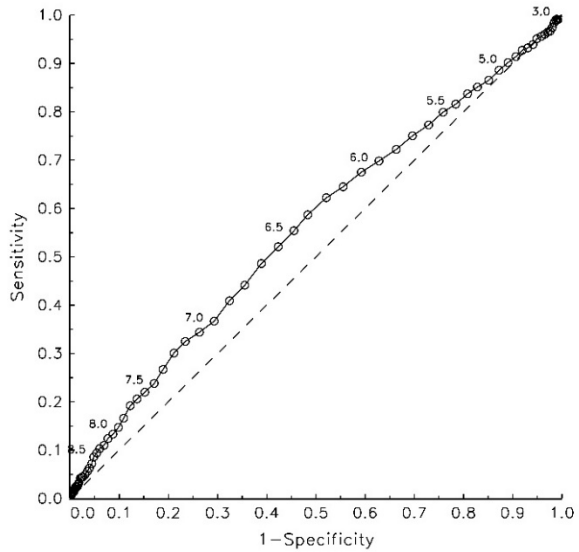

Figure 3.
The overall ability of glucose to predict large-for-gestational-age births.

The demographic maternal and infant characteristics of the development and validation groups were similar, but by chance there were significantly more women with BMI over 35, and significantly more SGA infants, in the development dataset than in the validation dataset.

Table 8 shows the odds ratios for LGA obtained from univariate and multiple logistic regression analyses based on the development sample. In the univariate analysis, all the factors evaluated except height (p = 0.0831, not shown) and parity ≥ 4 were significantly associated with LGA. In the first multiple model (including all the significant variables), all variables except maternal age remained significant. In the final multiple model, excluding maternal age, the factor most strongly associated with LGA was BMI ($p = 2.6 \times 10^{-19}$), accounting for 4.3% of the variance in the univariate setting (R^2 = 0.043). Using the validation database, the AUC for the final multiple model was 0.69 (95% CI 0.66–0.72), which was identical to the AUC retrieved from a model not including 2-h glucose (AUC =.0.69, 95% CI 0.66–0.72), and larger than from a model including 2-h glucose but not BMI (AUC = 0.63, 95% CI 0.60–0.67).

Table 8.
Risk factors for large-for-gestational-age infants in the development sample, using univariate and multiple logistic regression analysis

	Univariate model		Multiple model[a]		Final multiple model[b]		
Risk factor	**OR**	***p*-value**	**OR**	***p*-value**	**OR**	**95% CI**	***p*-value**
Maternal age (per 1-year increase)	1.04	0.005	1.01	0.677			
BMI (per 1-step increase)	1.11	< 0.001	1.10	< 0.001	1.10	1.08–1.13	< 0.001
2-h glucose (per 1 mmol increase)	1.12	0.003	1.09	0.033	1.09	1.01–1.18	0.028
Smoker	0.31	< 0.001	0.29	< 0.001	0.29	0.16–0.52	< 0.001
Parity 1	0.48	< 0.001	0.52	< 0.001	0.51	0.40–0,67	< 0.001
Parity ≥ 4	0.98	0.917					

[a] Multiple model included variables with $p < 0.05$ in univariate model.
[b] Final multiple model included variables with $p < 0.05$ in primary multiple model.
BMI, body mass index; CI, confidence interval; OR, odds ratio.

The overall abilities of the three models developed in predicting LGA in the validation sample were illustrated using ROC curves (Figure 4). The figure clearly shows that the ROC curve based on the model including BMI, nulliparity, and maternal smoking was identical to that based on the model in which glucose levels were also added, whereas the performance of the model that included glucose levels but not BMI was considerably poorer.

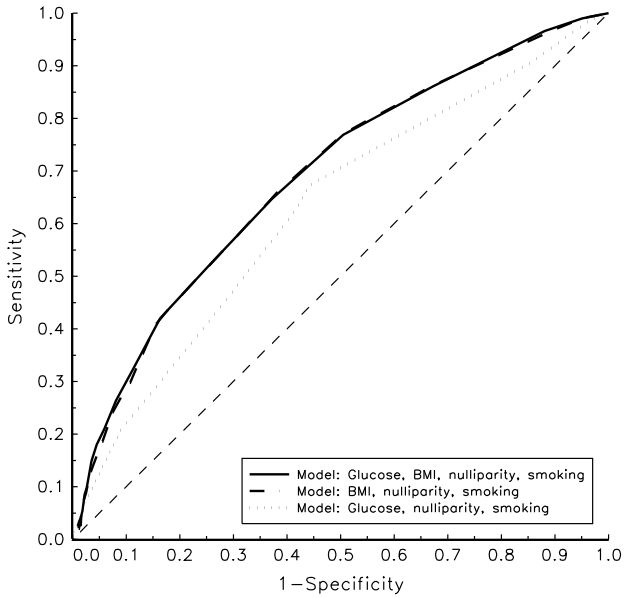

Figure 4.
ROC curves obtained after application of the three prediction models based on the validation data.

Paper IV. Prediction of postpartum diabetes with HbA1c assessed during OGTT in pregnancy

Of the 391 women who agreed to participate prospectively, 5-year data were available for 196 of them. Among these, 73% were of European origin (mostly Swedish) and 27% were of non-European origin (with Arab and Asian origin being the largest groups).

Mean values (SD) for maternal age, diagnostic 2-h plasma glucose concentration, and HbA1c level during pregnancy in participants were 33.3 (4.9) years, 11.1 (1.7) mmol/l, and 33.1 (7.1) mmol/mol [5.2% (1.1%)], respectively. The corresponding figures for non-participants were 32.4 (5.8) years, 11.0 (1.1) mmol/l, and 32.7 (5.8) mmol/mol [5.1% (0.9%)], and the differences compared to participants were not significant. After five years, 73 women had been diagnosed with diabetes: 14 before the first follow-up, 25 at the first (1- to 2-year) follow-up, 13 between the first follow-up and the final (5-year) follow-up, and 21 at the final follow-up. Of the remaining

123 women who participated in the 5-year follow-up (out of a total of 144), 60 were classified as having NGT and 63 were classified as having IFG/IGT (pre-diabetes).

The mean HbA1c level during pregnancy in women who had developed diabetes after 5 years was 36.7 (95% CI: 34.5–38.8) mmol/mol [5.5% (5.3–5.7%)], as compared to 31.4 (30.4–32.4) mmol/mol [5.0% (4.9–5.1%)] in women with pre-diabetes and 30.6 (29.5–31.7) mmol/mol [4.9% (4.8–5.1%)] in women with NGT at 5 years ($p < 0.0001$).

Using NGT at 5-year follow-up as a reference, an ROC curve was constructed to evaluate HbA1c as a predictor of diabetes up to five years after pregnancy (Figure 5). The ability of the ROC curve to predict diabetes was fair (AUC = 0.720, 95% CI 0.634–0.806; $p < 0.0001$), with an optimal cut-off point of 36 mmol/mol (5.4%), resulting in a sensitivity of 45% and a specificity of 92%. Table 9 shows the sensitivity, specificity, PPV, and NPV for various cut-offs. Overall, HbA1c showed high specificity and high PPV, but the sensitivity was low. The prediction did not improve by using both NGT and IFG/IGT at 5-year follow-up as a reference (AUC = 0.710, 95% CI 0.630–0.791; $p < 0.0001$). Similar results were obtained when we included women of Nordic origin only (diabetes, n = 23 vs. NGT, n = 44; AUC = 0.734, 95% CI 0.588–0.879; $p = 0.002$).

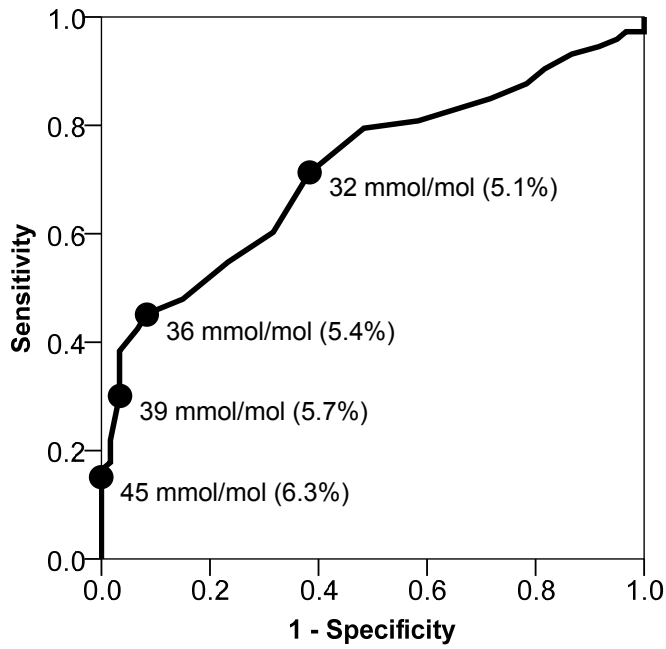

Figure 5.
Predictive accuracy of HbA1c in detecting diabetes five years after gestational diabetes, using women with normal glucose tolerance as a reference. Various cut-off points are shown.

Table 9.
Diagnostic indices of various HbA1c thresholds to predict diabetes five years after pregnancy using normal glucose tolerance at 5-year follow-up as a reference

HbA1c cut-off	n[a]	Sensitivity, %	Specificity, %	PPV, %	NPV, %
≥ 48 mmol/mol (≥ 6.5%)	10	13.7	100.0	100.0	48.8
≥ 45 mmol/mol (≥ 6.3%)	12	16.4	100.0	100.0	49.6
≥ 42 mmol/mol (≥ 6.0%)	15	19,2	98.3	93.3	50.0
≥ 39 mmol/mol (≥ 5.7%)	24	30.1	96.7	91.2	53.2
≥ 36 mmol/mol (≥ 5.4%)	38	45.2	91.7	86.8	57.8
≥ 32 mmol/mol (≥ 5.1%)	75	71.2	61.7	69.3	63.8

[a]Number of women who reached the threshold value.
NPV, negative predictive value; PPV, positive predictive value.

In Figure 6, HbA1c levels are plotted against the diagnostic 2-h capillary plasma glucose concentrations during pregnancy for the whole study group. After five years, all ten women with HbA1c levels ≥ 48 mmol/mol (≥ 6.5%) had been diagnosed with diabetes: six women before the first follow-up (HbA1c 51–70 mmol/mol [6.8–8.6%]), one woman at the first follow-up (HbA1c 57 mmol/mol [7.4%]), and three women at the five-year follow-up (HbA1c 50–55 mmol/mol [6.7–7.2%]). Similarly, in 13 women with HbA1c levels ≥ 45 mmol/mol (≥ 6.3%), all but 1 woman (IGT) had been diagnosed with diabetes after five years. Altogether, five out of 27 women with HbA1c levels ≥ 39 mmol/mol (≥ 5.7%) had not been diagnosed with diabetes after five years (2 NGT, 1 IFG, and 2 IGT). The corresponding value for women with 2-h capillary plasma glucose levels of ≥ 12.2 mmol/l (the diagnostic limit for diabetes outside of pregnancy) was eight out of 24 (1 NGT, 3 IFG, and 4 IGT).

HbA1c levels for the total study group were grouped into quartiles. Median levels for HbA1c in mmol/mol [%] in the respective quartiles were: 27 (range: 21–29) [4.6% (range 4.1–4.8%)] (n = 56), 31 (30–31) [5.0% (4.9–5.0%)] (n = 43), 33 (32–35) [5.2% (5.1–5.4%)] (n = 51), and 40 (36–70) [5.8% (5.4–8.6%)] (n = 46). A logistic regression analysis, testing the predictive value of HbA1c quartiles for the 5-year diabetes risk, showed that women with HbA1c levels in quartile four had a sevenfold higher risk of postpartum diabetes than women with HbA1c levels in quartiles 1–3 (OR = 7.0, 95% CI 3.3–14.6; $p < 0.0001$). This association remained significant after adjustment for maternal age and the 2-h glucose level during pregnancy (OR = 5.5, 95% CI 2.5–12.1; $p < 0.0001$).

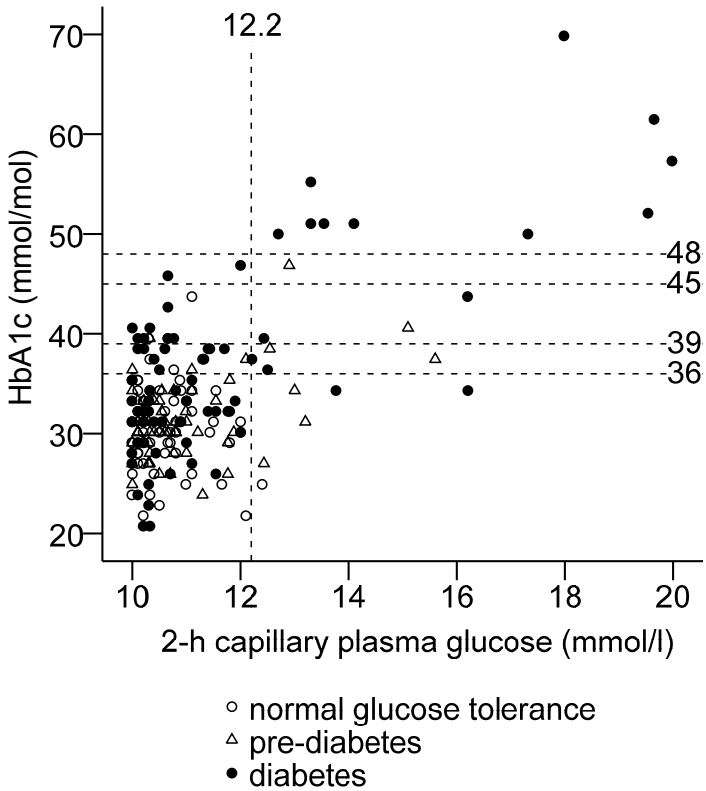

Figure 6.
HbA1c levels plotted against the diagnostic 2-h glucose concentration during pregnancy in 196 women with gestational diabetes. Various diagnostic cut-off levels are shown, and the diagnoses at the 5-year follow-up are indicated by symbols.

Paper V. Seasonality of GDM

Of the 11,538 women who underwent an OGTT during the study period, 487 women (4.2%) were diagnosed with GDM.

Table 10 shows the study material, organized by month and season. The monthly frequency of GDM ranged from 2.9% in March to 5.8% in June, and the seasonal frequency of GDM ranged from 3.3% in spring to 5.5% in summer. The differences in frequencies were statistically significant, both for month ($p = 0.01$) and for season ($p < 0.0001$). The mean age of participating women was 29.9 (SD 5.1) years, and the ages ranged from 15 to 49 years. The age of the women differed statistically significantly between months and seasons ($p < 0.001$). However, no significant differences in the monthly distributions of age were noted.

Table 10.
Description of the study material according to month and season

		OGTT n	GDM n (%)	2-h glucose, mmol/l Mean (SD)	Age, years Mean (SD)	Temperature, °C Mean
Month						
	January	1,094	36 (3.3)	6.43 (1.26)	30.4 (5.0)	0
	February	928	34 (3.7)	6.39 (1.25)	30.1 (5.3)	−0.6
	March	1,082	31 (2.9)	6.41 (1.22)	30.1 (5.2)	2.4
	April	1,027	34 (3.3)	6.52 (1.26)	30.1 (5.0)	7.5
	May	1,057	41 (3.9)	6.49 (1.35)	30.4 (5.1)	12.1
	June	1,009	59 (5.8)	6.60 (1.44)	29.8 (5.1)	15.2
	July	974	50 (5.1)	6.55 (1.33)	30.0 (5.0)	17.7
	August	928	52 (5.6)	6.61 (1.33)	29.6 (5.0)	17.6
	September	781	33 (4.2)	6.54 (1.27)	29.5 (5.3)	14.4
	October	835	42 (5.0)	6.59 (1.32)	29.6 (5.1)	8.4
	November	897	38 (4.2)	6.60 (1.32)	29.6 (5.2)	5.3
	December	926	37 (4.0)	6.50 (1.28)	29.8 (5.1)	2.9
Season						
	Winter	2,948	107 (3.6)	6.44 (1.26)	30.1 (5.1)	0.7
	Spring	3166	106 (3.3)	6.47 (1.28)	30.2 (5.1)	7,3
	Summer	2911	161 (5.5)	6.59 (1.37)	29.8 (5.0)	16.8
	Autumn	2513	113 (4.5)	6.58 (1.31)	29.6 (5.2)	9.2

GDM, gestational diabetes mellitus; n, number; OGTT, oral glucose tolerance test; SD, standard deviation.

Mean monthly temperature ranged from −0.6°C in winter to 17.7°C in summer (Table 10). In a simple linear regression with 2-h plasma glucose as the dependent variable and mean monthly temperature as the predictor variable, the coefficient in the equation was 0.009, suggesting that the 2-h glucose level increased by 0.009 mmol/l for every degree increase in temperature ($p < 0.0001$).

Figure 7 illustrates the monthly mean 2-h glucose level during the OGTT (with 95% CI) and the monthly percentage of women with GDM. Though numerically small, the differences in 2-h glucose levels were statistically significant ($p < 0.001$), with the lowest values observed from January to March and peak levels from June to August. A similar seasonal trend was seen in the percentage of women with 2-h glucose levels in the GDM range (2-h glucose level ≥ 8.9 mmol/l). There were no significant differences in the distribution of glucose concentrations between months or seasons.

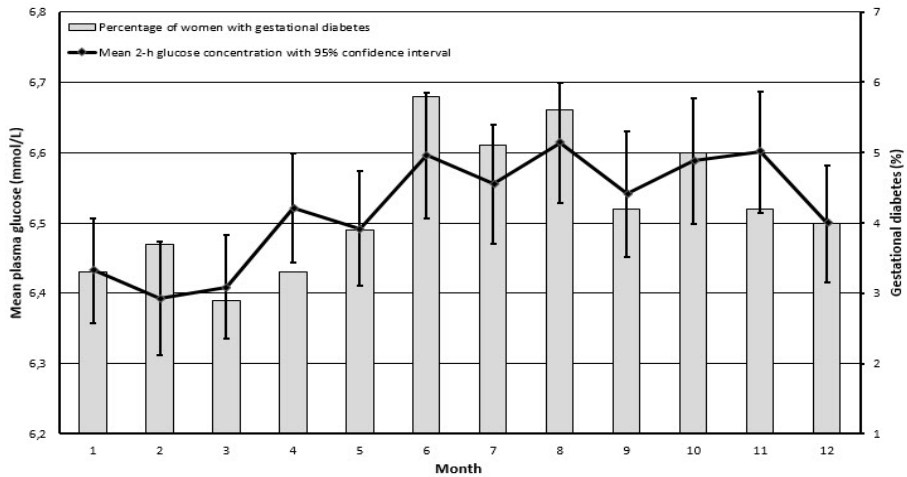

Figure 7.
Monthly mean 2-h glucose levels and the monthly percentages of women with gestational diabetes mellitus.

In regression analysis, adjusting for age, the summer months (June to August) were found to be associated with increased 2-h glucose levels ($p < 0.001$) and increased frequency of GDM compared to all other months (OR = 1.51, 95% CI 1.24–1.83; $p < 0.001$).

Discussion

The main focus of this thesis is diagnostics. The questions raised in the different studies can be divided into three main categories: diagnostics of GDM during pregnancy (Papers I and V), diagnostics of type-2 diabetes at follow-up after GDM (Papers II and IV), and the relative contributions of maternal BMI and glucose levels in the prediction of LGA births (Paper III).

Diagnostics of GDM during pregnancy

Our findings showed a modest increase of 26% in the number of women diagnosed with GDM using the IADPSG criteria. This could be explained by selection bias, because all the women invited to take part in the present study were included on the basis of their 2-h capillary glucose value. In line with this, the number of women identified by the 2-h glucose value was disproportionally high (79%) compared to the HAPO study (38%) (139).

The lack of fasting glucose measurement in the screening procedure may partly explain the relatively low increase in the number of women identified as having GDM by the WHO criteria, and particularly, the IADPSG criteria. Based on the present findings, the number of women diagnosed as having GDM in southern Sweden using a 2-h capillary glucose concentration of ≥ 10.0 mmol/l as a selection criteria for a diagnostic OGTT, would hypothetically increase from 2.6% to 3.3% if the IADPSG criteria were applied (75). These criteria may possibly have less impact on a low-risk population like the Swedish one. Furthermore, our population sample was historical, and may not have been representative of the contemporary population. For comparison, in a recent study performed in Norway, including 59% from ethnic minority groups, a 2.4 times increase in the prevalence of GDM was reported when applying the modified IADPSG criteria, compared to the WHO criteria; indicating an increase from 13.0% to 31.5%. The observed difference was mainly the result of the lower fasting glucose threshold in the IADPSG criteria (35). In the total HAPO cohort, the overall prevalence of GDM was 17.8% (139). However, a more modest increase has been reported in other populations (140-142).

From Paper I, it is obvious that some women will be missed if the fasting glucose level is not taken into account in the screening procedure.

In Paper V, we found seasonal variations in the 2-h glucose level in the OGTT performed in the twenty-eighth week of gestation, giving seasonal variations in the percentage of women diagnosed with GDM—with a peak in the summer.

Increased arterialization of the venous blood at elevated temperatures has been suggested to be a plausible explanation (143). Whether these variations in glucose levels result from an acute effect of temperature rather than a chronic one is not fully understood, although some experimental studies have indicated an acute effect (144, 145). Since the study in Paper V was based on capillary glucose measurements, representing a mixture of arterial and venous blood, temperature-induced changes may very well affect the composition of capillary blood as well.

There have been previous studies with results that support our findings (80-82), but on the other hand there are others that did not show any seasonality in glucose tolerance or in the incidence of GDM (83, 84).

Worsening of metabolic control in subjects with type-2 diabetes in winter has been described in a number of studies (146-149). Since diet and exercise are hallmarks of the treatment of type-2 diabetes, it is reasonable to assume that environmental factors, such as diet and exercise patterns, have an important role in the seasonal variation in glucose metabolism in patients with diabetes. Seasonal variation in the diagnosis of GDM possibly reflects seasonality of environmental influences early in gestation, during placental development, affecting placental metabolism and glucose homeostasis later on in pregnancy (81). Many factors vary with season, including the nutritional quality of foods, temperature, the number of hours of sunshine, and vitamin D synthesis. Maternal vitamin D deficiency in early pregnancy has been associated with increased risk of GDM (78). Moreover, seasonal variation in vitamin D status, quantified as the total number of hours of sunshine during the three months preceding the onset of diabetes, was suggested as an explanation for the seasonality of type-2 diabetes reported by Doró et al. (150).

Diagnostics of type-2 diabetes at follow-up after GDM

Paper II was the first study in Sweden to compare the performance of HbA1c with those of established glucose criteria during the OGTT in women with previous GDM. Proposed cut-off points of HbA1c showed suboptimal performance relative to the OGTT in the diagnosis of diabetes and abnormal glucose tolerance. Combined with a fasting glucose test, the diagnostic accuracy improved—although to an extent similar to that obtained using the fasting glucose test alone.

In line with previous studies, we found poor agreement in the consistency between HbA1c and OGTT criteria in classifying diabetes and abnormal glucose tolerance postpartum, although correlations between HbA1c and glucose values obtained during the OGTT indicated fairly good agreement (111, 114, 116, 151). A recent Norwegian study found that women with GDM had twice the risk of elevated HbA1c early postpartum (128).

The rationale for recommending OGTT postpartum in women with GDM is not only to detect women with apparent diabetes but also to identify women with pre-diabetes and IGT in whom diabetes can be delayed or prevented (97, 152). We therefore hypothesized that a reasonable screening model would be to accept all women with IFG for intensive follow-up and prevention without retesting—in our sample, corresponding to 35% of the study population (49/140). If one accepts HbA1c 5.0% (31 mmol/mol) as a cut-off for further identification, this would leave 59 women for confirmatory testing by OGTT, among whom 36% (21/59) would be diagnosed with diabetes or IGT based on the 2-h glucose value alone. Of the remaining 32 women, 25% would be misclassified as having normal glucose metabolism, i.e. 10% (8/78) of the women with any kind of abnormal glucose tolerance in the study cohort.

In addition to the 2-h plasma glucose concentration during pregnancy, we have recently reported that (1) BMI at the first follow-up after pregnancy and (2) having a non-European background were the most important risk factors for development of diabetes five years after pregnancy in the total Mamma study cohort (with GDM defined by the WHO 1999 criteria) (108). However, HbA1c was not included in the prediction model since it was only measured in women diagnosed with GDM according to clinical routine (EASD criteria).

In Paper IV, we were able to confirm our previous findings that HbA1c levels in the upper quartile, measured close to the diagnostic OGTT during pregnancy, predict diabetes development during the five years after delivery (107). To the best of our knowledge, only four other studies have investigated an association between HbA1c levels during pregnancy and the risk of postpartum diabetes (153-156).

In our material, an HbA1c level of ≥ 48 mmol/mol (≥ 6.5%) during the third trimester of pregnancy identified all women with a diabetes diagnosis five years after pregnancy, some of whom had been diagnosed with diabetes before the first follow-up and might have had pre-gestational diabetes. Furthermore, an HbA1c level of ≥ 45 mmol/mol (≥ 6.3%) identified all but 1 woman with diabetes after five years, and an HbA1c level of ≥ 39 mmol/mol (≥ 5.7%) identified all but 5 women with a diabetes diagnosis during follow-up. On the other hand, for the various thresholds, HbA1c had low sensitivity in diagnosing diabetes using either NGT or NGT/IFT/IGT as a reference. These data provide evidence to suggest there may be a useful HbA1c threshold above which all women should be closely monitored, starting already during pregnancy, to prevent diabetes development after delivery. Furthermore, after

adjustment for the 2-h glucose level, HbA1c levels equal to and above the optimal cut-off level of the ROC curve were associated with more than a 5-fold increased risk of postpartum diabetes. This indicates that HbA1c analysis could be an adjunct to the OGTT in identifying women who are at high risk of developing postpartum diabetes.

How different maternal measures affect pregnancy outcome

The main findings of the study presented in Paper III were that both the 2-h glucose level of the OGTT and maternal BMI had a significant effect on the risk of delivering an LGA neonate. However, the relative contribution of BMI was much higher, even when taking other risk factors into account. The overall ability of the developed model to predict LGA in the validation sample was satisfactory, but it was identical to that of a model that did not include the 2-h glucose level.

Based on the ROC curve of the total dataset, we found no apparent natural cut-off point above which there would be an increased risk of having an LGA infant. This is in line with the HAPO study, which showed that maternal hyperglycemia was associated with perinatal risk in a linear way, with no obvious threshold (41). Whereas all other guidelines for the diagnosis of GDM are more or less based on arbitrary statistics, the IADPSG criteria are based (for the first time) on perinatal outcomes (61, 157). From this, it is obvious that the simplified method, omitting the initial fasting glucose sample during the OGTT, is not optimal for prediction of the gestational weight of a newborn.

We have previously shown that maternal characteristics such as age, parity and smoking, in addition to BMI and maternal glucose status, influence fetal growth during the last trimester (158). The logistic regression modeling identified the independent variables available from the register that are important and that can help in the prediction of LGA births.

Overall, the associations between maternal pre-pregnancy obesity and adverse pregnancy outcomes appear to be stronger than those between excessive GWG and adverse pregnancy outcomes (159), although some studies have indicated that GWG is of greater importance (160, 161).

Some recent studies have suggested that HbA1c can be used to predict adverse outcomes in pregnancy, especially LGA and macrosomia (162-165). However, other researchers have not been able to confirm these results (166, 167). It would have been of great interest to analyze the relationship between HbA1c and LGA in our pregnant population, but unfortunately HbA1c is not measured on a regular basis during pregnancy in southern Sweden.

Conclusions

The main conclusions drawn from these studies are summarized below.

Paper I:

Twenty-six per cent more women were identified by the IADPSG criteria and 20% more women were identified by the WHO criteria, compared to the criteria presently employed.

A greater increase may be expected in an unselected pregnant population.

Paper II:

Proposed thresholds of HbA1c (≥ 48 mmol/mol [≥ 6.5%] and ≥ 39 mmol/mol [≥ 5.7%]) had low sensitivity in diagnosis of diabetes and abnormal glucose tolerance in this study cohort.

In combining HbA1c with a fasting glucose test, the performance was no better than using a fasting glucose test alone.

Considering that early detection of pre-diabetes is of utmost importance in these women to prevent the development of diabetes, combining a fasting glucose test with a lower cut-off point of HbA1c may be an alternative approach to select women for an OGTT and identify those who have isolated post-glucose load hyperglycemia.

With an HbA1c cut-off of ≥ 31 mmol/mol (≥ 5.0%), the number of women who would need a confirmatory OGTT decreased by almost 60%, thus overlooking 10% of those with abnormal glucose tolerance in the study cohort.

Paper III:

Maternal BMI had a greater impact on the prediction of LGA births than the 2-h glucose level of the OGTT.

The data highlight the importance of targeting healthy body weight in pregnant women and closer monitoring of weight during pregnancy as a strategy for reducing the risk of excessive fetal growth.

Paper IV:

An HbA1c level of ≥ 36 mmol/mol (≥ 5.4%), obtained close to the twenty-eighth week of pregnancy, was associated with a more than fivefold increased risk of diabetes five years after pregnancy.

A cut-off level for HbA1c of ≥ 39 mmol/mol (≥ 5.7%), corresponding to the pre-diabetes range outside of pregnancy, could reveal women with postpartum diabetes with high specificity (97%) and high PPV (91%).

Due to the low sensitivity, HbA1c does not appear to be suitable as a screening test to predict diabetes after GDM in all women, but it could be used as a strategy for selecting high-risk women for lifestyle interventions to prevent diabetes, starting already in pregnancy.

Paper V:

Based on a universally performed OGTT in the twenty-eighth week of pregnancy, seasonality in the proportion of women diagnosed with GDM was observed, with a peak in the summer.

The mean 2-h glucose concentration in the OGTT followed the same seasonal trend.

The findings may be related to the increased ambient temperature in the summer.

Reflections for future work

Due to the lack of a uniform diagnostic procedure for the diagnosis of GDM in Sweden, there is no accurate estimate of the national prevalence of GDM. Today, about 2–3% of pregnant women are diagnosed with GDM (75, 76). Based on figures from the HAPO cohort, an increase to 15–20% can be expected if the IADPSG criteria based on universal OGTT are applied (139). Nevertheless, since the HAPO study was an observational study and not a treatment one, the question remains as to whether treatment can reduce GDM-associated complications enough to make diagnosis based on IADPSG criteria clinically relevant and cost-effective.

To date, there have been no studies evaluating the effects of treatment at the population level based on the diagnostic glucose thresholds proposed by the IADPSG and the WHO (8, 61). In 2015, the Swedish National Board of Health and Welfare reviewed the evidence for the current Swedish criteria and the new WHO criteria and recommended a move to the new WHO thresholds, from which treatment should be initiated (67). The board recommended that every county council should include these criteria in their clinical guidelines and decide how to implement the relevant changes locally. With the ambition of enabling all the Swedish maternity clinics to implement a national standardized and harmonized practice in screening and diagnosis of GDM, a national prospective, stepped wedge randomized controlled trial has been planned, the CDC4G (Changing Diagnostic Criteria for Gestational diabetes) trial. The initiative for the study came from the expert panel in the National Board of Health working group for the new guidelines. The hypothesis is that treating women with GDM, defined by the new thresholds, will reduce adverse pregnancy outcomes in the Swedish population. Rates of neonatal and maternal outcomes before and after the change will be compared along with a health cost analysis. The National Pregnancy Register, which collects data on all pregnancies in Sweden, will be expanded and constitute the source of data for the study. The study cohort will be followed prospectively to compare the long-term consequences for the mother and the child by linkage to the National Diabetes Register and the Child Health Register. The project will be launched in September 2017. Depending on the number of participating maternity centers, the first results will be available within 1 to 2 years from the start of the study. The screening procedure (universal or selective) will be unchanged during the recruitment period, but guidelines for treatment and obstetrical surveillance will be uniform and well implemented in all centers before the start of the study.

The CDC4G trial will allow further evaluation of the questions raised in this thesis, based on the extensive material made available through the Pregnancy Register.

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Paper I

AOGS
ACTA Obstetricia et Gynecologica Scandinavica

AOGS SHORT RESEARCH REPORT

The potential impact of new diagnostic criteria on the frequency of gestational diabetes mellitus in Sweden

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Abstract

The International Association of Diabetes and Pregnancy Study Groups (IADPSG) has suggested new diagnostic criteria for gestational diabetes mellitus. Many centers in Europe still use the World Health Organization (WHO) criteria. In southern Sweden we use the 2-h threshold of the European Association for the Study of Diabetes criteria based on universal screening with a 75-g oral glucose tolerance test. We have retrospectively scrutinized oral glucose tolerance tests in a subset of 174 women included in a previous study, diagnosed with gestational diabetes mellitus 1996–1999. A complete repeat oral glucose tolerance test was performed directly after diagnosis in 120 women. When applying the current Swedish criteria, and the IADPSG and the WHO criteria to the material, gestational diabetes mellitus was confirmed in 67% (80/120), 84% (101/120), and 80% (96/120), respectively. Hence, 26% (101/80) more women were identified by the IADPSG criteria and 20% (96/80) more women by the WHO criteria, compared with the criteria presently in use.

Abbreviations: EASD, European Association for the Study of Diabetes; GDM, gestational diabetes mellitus; HAPO, Hyperglycemia and Adverse Pregnancy Outcome study; IADPSG, International Association of Diabetes and Pregnancy Study Groups; OGTT, oral glucose tolerance test; WHO, World Health Organization.

Introduction

It is internationally agreed that the diagnosis of gestational diabetes mellitus (GDM) should be based on a 75-g oral glucose tolerance test (OGTT), but there is lack of consensus regarding the screening procedure and diagnostic thresholds (1,2). Recently, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) proposed new guidelines for the diagnosis of GDM based on results from the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study, prescribing lower diagnostic thresholds than those currently used in most parts of the world (3).

The impact of the new IADPSG criteria on the frequency of GDM depends on the population under consideration and the current guidelines. In Europe, either the World Health Organization (WHO) criteria (2), or the European Association for the Study of Diabetes (EASD) criteria (4) are most frequently used when diagnosing GDM. Compared with these criteria, the IADPSG criteria prescribe a lower threshold for the fasting glucose value, whereas the 2-h cutoff value is higher than in the WHO criteria, but similar to the EASD criteria.

Using the slightly modified EASD criteria, defining GDM as a 2-h capillary blood glucose concentration of ≥9.0 mmol/L during a universal 75-g OGTT, the prevalence of GDM in southern Sweden has been 1.9% (5). Here, we present the results of a pilot study carried out to obtain information on how the introduction of the new IADPSG criteria would affect the prevalence of GDM

in our region. The effect of applying the WHO criteria was also evaluated.

Material and methods

In 1995, a general screening program for GDM was introduced in southern Sweden offering a 75-g OGTT at the local antenatal clinic to all women in gestational week 28, and also in week 12 if they had a first-degree family history of diabetes or GDM in previous pregnancies. GDM was defined as a 2-h capillary blood glucose concentration of ≥9 mmol/L (4,5). If glucose concentrations were between 7.8 and 8.9 mmol/L the OGTT was repeated within 1 week, and if normal no more measures were taken. The laboratory procedure using HemoCue blood glucose meters (HemoCue, Ängelholm, Sweden) has shown a coefficient of variation of 3.1–3.7% (5). In 2004, glucose measurements in Sweden switched from blood to plasma glucose and a HemoCue glucose meter was introduced (HemoCue 201+ system) converting blood glucose concentrations to equivalent plasma glucose concentrations by multiplying by a constant factor of 1.11. According to a recently presented conversion algorithm, the resulting capillary 2-h threshold value of 10.0 mmol/L corresponds to venous plasma glucose 8.5 mmol/L (6). To enable comparison with the IADPSG and the WHO criteria, this was the 2-h threshold used in the present study.

All women diagnosed with GDM in the region of Malmö and Trelleborg in southern Sweden are referred to the Department of Endocrinology in Malmö for follow-up during pregnancy. Women referred between 1996 and 1999 were invited to take part in a 5-year follow-up study, including a repeat OGTT after overnight fasting as soon as possible (median 9 days, interquartile range 6 days) after referral (7). Venous samples were drawn at 0, 60, and 120 min, and immediately analyzed in a HemoCue blood glucose meter for the determination of glucose concentration. Weight and height were recorded, and the body mass index was calculated.

Out of 188 consecutive women, 182 agreed to take part in the follow-up study. Eight of these 182 women were not included in the analysis because a repeat OGTT could not be performed at the start of the study. Of the 174 women remaining, at least one glucose value was missing during the OGTT in 54 of them. Hence, 120 women underwent a complete repeat OGTT: mean (SD) age 31.6 (5.4) years, and body mass index 28.7 (5.0) kg/m^2. Half of them were of non-Nordic origin.

Women in whom the GDM diagnosis was consistent with the modified EASD criteria were identified and the additional number of women identified when applying the IADPSG criteria to this group was determined.

Table 1. Threshold values for the diagnosis of gestational diabetes mellitus according to the different criteria.

	Venous plasma glucose concentration threshold (mmol/L)[a]		
Criteria	Fasting	1-h	2-h
Modified EASD	NA	NA	≥8.5
IADPSG	≥5.1	≥10.0	≥8.5
WHO	≥7.0	NA	≥7.8

[a]One or more of these values must be equaled or exceeded for the diagnosis of gestational diabetes mellitus.
EASD, European Association of the Study of Diabetes; IADPSG, International Association of the Diabetes and Pregnancy Study Groups; WHO, World Health Organization; NA, not applicable.

Similarly, the number of women identified as having GDM using the WHO criteria was calculated. The diagnostic threshold values prescribed by the different criteria are presented in Table 1.

Informed consent was obtained from the participants, and the Ethics Committee of Lund University approved the study protocol (LU112-96).

Results

Based on a 2-h plasma glucose threshold of 8.5 mmol/L, GDM was confirmed in 67% (80/120) of the women (Table 2). Including the value of the fasting plasma glucose, according to the IADPSG criteria, identified an additional 5.0% (6/120), and including the 1-h plasma glucose value identified another 13% (15/120). Three of the women identified by the modified EASD criteria had a fasting glucose level ≥7.0 mmol/L and one of the women not identified by these criteria had a fasting glucose level above this threshold.

Hence, when applying the IADPSG criteria to the whole study group, 84% (101/120) were diagnosed as having GDM: 80/101 fulfilled the criteria for the 2-h plasma glucose level, 47/101 fulfilled the criteria for the fasting plasma glucose level, and 64/101 fulfilled the criteria for the 1-h plasma glucose level. Accordingly, 79% (80/101) of the women identified by the IADPSG criteria were also identified as having GDM by the modified EASD criteria.

Based on the WHO criteria, 80% (96/120) were diagnosed as having GDM, all on the basis of the 2-h threshold value. Of the 101 women identified by the IADPSG criteria, 93 were also identified by the WHO criteria. Seven of those not identified as having GDM by the WHO criteria had a 1-h plasma glucose concentration of ≥10.0 mmol/L and one had a fasting plasma glucose concentration of 5.8 mmol/L. In addition, three of the

Table 2. Frequency of confirmed diagnosis of gestational diabetes mellitus and subjects with glucose values above specific thresholds.

		n/total (%) GDM diagnosed considering each glucose level sequentially[a]			*n*/total (%) GDM diagnosed considering individual glucose levels[b]		
Criteria	*n*/total (%) GDM diagnosis confirmed	2-h PG*	FPG**	1-h PG*	FPG**	1-h PG*	2-h PG*
Modified EASD	80/120 (67)	80/80 (100)	NA	NA	NA	NA	80/80 (100)
IADPSG	101/120 (84)	80/101 (79)	6/101 (6)	15/101 (15)	47/101 (47)	64/101 (63)	80/101 (79)
WHO	96/120 (80)	96/96 (100)	0/96 (0)	NA	4/96 (4)	NA	96/96 (100)

[a]Additional number of women identified by each threshold starting with the 2-h PG.
[b]Number of women identified by each glucose threshold.
PG, plasma glucose; FPG, fasting plasma glucose; GDM, gestational diabetes mellitus; EASD, European Association of the Study of Diabetes; WHO, World Health Organization; IADPSG, International Association of the Diabetes and Pregnancy Study Groups; NA, not applicable.

women who did not meet the IADPSG criteria had 2-h plasma glucose concentrations between 7.8 and 8.5 mmol/L.

The IADPSG criteria identified 26% (101/80) and the WHO criteria identified 20% (96/80) more women as having GDM than the modified EASD criteria.

Discussion

In line with the present recommendation by the IADPSG, universal screening with a 75-g OGTT has been performed in southern Sweden since 1995. To increase compliance with the program a simplified OGTT is used, omitting the initial fasting glucose sample (5). This decision was supported by a study indicating that fasting glucose concentrations do not increase in normal pregnancy and have low sensitivity in detecting GDM (8). However, there has been some criticism of this simplification, and we lack information on how many women are missed by not taking the fasting glucose threshold into account.

Another simplification employed in the screening procedure is the use of capillary glucose samples, measured on-site using a patient-near method. Although the method is not regarded as a diagnostic standard, capillary glucose samples and the HemoCue glucose meter are widely used for diagnostic purposes in Sweden (1,3). To allow comparisons between the different diagnostic criteria, the capillary 2-h threshold was converted to its venous counterpart using a recently presented algorithm (6). The resulting value of 8.5 mmol/L coincides with the 2-h threshold proposed by the IADPSG, facilitating comparison.

The diagnosis of GDM was confirmed in only 80 of the 120 women by the criteria presently in use and this could have several explanations, but first and foremost the well-known variability and low reproducibility of the OGTT; the variability of the 2-h value being especially high (9). The use of HemoCue devices made it possible to obtain an immediate diagnosis of GDM for optimal patient information and care. For this reason it can be assumed that some changes in lifestyle had taken place during the days that elapsed between the diagnostic test and the second OGTT. Furthermore, the applied conversion algorithm did not show perfect agreement, which may exert an influence on the results (6).

In addition to the 80 women identified by the 2-h threshold value of the IADPSG criteria, the fasting and the 1-h threshold values identified another 21 women. Using the individual plasma glucose thresholds of the IADPSG criteria: fasting, 1-h, and 2-h, identified 47, 63 and 79% of the women, respectively. The corresponding values in the total HAPO cohort were 55%, 55%, and 38%, although there was substantial variation between centers: 24–74, 32–76 and 26–65% (10). Nevertheless, taking this variation into account, the number of women identified by the 2-h glucose value in our study group is disproportionately high. This could be explained by a selection bias, because all women invited to take part in the present study were included on the basis of their 2-h glucose value.

The lack of fasting glucose measurement in the screening procedure may also partly explain the relatively low increase in the number of women identified as having GDM by the WHO criteria and, in particular, the IADPSG criteria. Based on the present findings, the number of women diagnosed as having GDM in southern Sweden would hypothetically increase from 1.9 to 2.4% if the IADPSG criteria were applied (5). Possibly, these criteria may have less impact on a low-risk population like the Swedish. Furthermore, our population sample was historical and may not be representative of the contemporary population. For comparison, in a recent study performed in Norway, including 59% from ethnic minority groups, a 2.4 times increase in the prevalence of GDM was reported when applying the modified IADPSG criteria, compared with the WHO criteria; implying an increase from 13.0 to 31.5%. The observed difference was mainly the result of the lower fasting glucose threshold in the

IADPSG criteria (11). In the total HAPO cohort the overall prevalence of GDM was 17.8% (10). However, a more modest increase has been reported in other populations (12).

The impact of applying the IADPSG criteria to a given population depends on the diagnostic guidelines being used. Most regions in Sweden have adopted a capillary 2-h plasma glucose concentration of ≥10.0 mmol/L for the diagnosis of GDM, and/or a fasting plasma glucose concentration of ≥7.0 mmol/L. From the present study it is obvious that some women will be missed if the fasting glucose level is not taken into account in the screening procedure. On the other hand, in other parts of Sweden, repeated random plasma glucose measurements are frequently used to identify women for an OGTT. We have previously shown that universal screening with OGTT detects twice as many women with GDM as selective screening with random plasma glucose measurements (5). Furthermore, the screening procedure in southern Sweden is optimized by offering a repeat OGTT to women with 2-h glucose concentrations in the intermediate range. The low reproducibility of the OGTT, particularly the 2-h values in the near-normal range, supports the suitability of this procedure (9).

In conclusion, 26% more women were identified by the IADPSG criteria and 20% more women by the WHO criteria, compared with the criteria presently employed. A greater increase may be expected in an unselected pregnant population. The results emphasize the need for a prospective study to determine the true increase in the prevalence of GDM resulting from the introduction of IADPSG guidelines in Sweden.

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Paper II

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Research Paper

Role of HbA1c in post-partum screening of women with gestational diabetes mellitus

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ABSTRACT

Aim: To compare the performance of HbA1c with established glucose criteria during an oral glucose tolerance test (OGTT) and to assess HbA1c as a screening test for undiagnosed diabetes and pre-diabetes after gestational diabetes mellitus (GDM).
Methods: Glucose homeostasis was re-evaluated 1–5 years after delivery in 140 women with previous GDM, by means of OGTT and simultaneous HbA1c measurement. Glucose tolerance was defined according to World Health Organisation criteria. HbA1c ≥6.5% (≥48 mmol/mol) was used for diabetes diagnosis and HbA1c ≥5.7% (≥39 mmol/mol) to define abnormal glucose homeostasis.
Results: HbA1c had low sensitivity (14.3%) and high specificity (99.1%) in diabetes diagnosis. Sensitivity and specificity of HbA1c to detect abnormal glucose tolerance were 29.5% and 95.2%, respectively. The consistency in classifying abnormal glucose tolerance between HbA1c and OGTT criteria was 59% ($\kappa = 0.227$) and the area under the receiver operating characteristic curve was 0.708. The combined use of HbA1c and fasting glucose criteria showed similar performance to that of fasting glucose criteria alone. The latter identified 63% of the women with pre-diabetes or diabetes in the study cohort. However, by lowering the cut-point of HbA1c to ≥5.0% (≥31 mmol/mol), an additional proportion (27%) with isolated post-glucose load hyperglycaemia was identified.
Conclusion: Proposed thresholds of HbA1c had low diagnostic sensitivity. Combined with a fasting glucose test, the performance was no better than with using a fasting glucose test alone. Combining a fasting glucose test with a lower HbA1c cut-point may be an alternative approach for selection of women for an OGTT.

Introduction

HbA1c has recently been approved by the World Health Organisation (WHO) as an alternative to the oral glucose tolerance test (OGTT) for the diagnosis of diabetes mellitus outside pregnancy [1]. A diagnostic cut-point of ≥6.5% (≥48 mmol/mol) was recommended based on the risk of developing microvascular complications such as retinopathy. No formal recommendations on the interpretation of HbA1c levels below this cut-point were made. However, the International Expert Committee (IEC) recommended that high-risk individuals with HbA1c levels between 6.0% (42 mmol/mol) and 6.4% (47 mmol/mol) should be considered for diabetes prevention and interventions [2], and the American Diabetes Association (ADA) suggested that HbA1c levels between 5.7% (39 mmol/mol) and 6.4% (47 mmol/mol) indicate intermediate hyperglycaemia [3].

Women with gestational diabetes mellitus (GDM) are a high-risk group for development of type-2 diabetes [4]. According to Swedish national guidelines, lifestyle intervention and follow-up of these women after pregnancy should have high priority, but it is not clear which measures should be followed. In the primary care setting, the

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use of HbA1c for screening and diagnostic purposes would be practical, possibly in combination with a fasting glucose test. Both are quick and easy to perform, are more convenient for and acceptable to patients, and are less expensive than the OGTT [5].

Using the slightly modified European Association for the Study of Diabetes criteria, defining GDM as a 2-h capillary blood glucose concentration of ≥9.0 mmol/l during a universal 75-g OGTT [6], the estimated prevalence of GDM in southern Sweden over the past decade has increased from 1.9 to 2.6% [7]. In a previous study from our area, it was reported that 30% of the women with GDM in the study cohort had developed diabetes 5 years after delivery [8]. Furthermore, fasting blood glucose levels of ≥5.2 mmol/l and HbA1c levels of ≥5.7% (≥38 mmol/mol) during pregnancy were found to be associated with a four-to six-fold increased risk.

The aim of the present study was to compare the performance of HbA1c testing with that of established glucose criteria during the OGTT at 1- to 5-year follow-up post-partum in this historical cohort of women with GDM, and to assess HbA1c as a screening test (alone or combined with a fasting glucose test) for undiagnosed diabetes and abnormal glucose tolerance.

Material and methods

All women who are diagnosed with GDM in the region of Malmö and Trelleborg in southern Sweden are referred to the Department of Endocrinology in Malmö for follow-up during pregnancy. Women referred between 1996 and 1999 were invited to take part in a 5-year follow-up program, including measurement of HbA1c and a 75-g OGTT at 1, 2, and 5 years after delivery. The study design has been described previously in detail [8]. Of 182 eligible women, a total of 174 were finally included. Only women with complete glucose data at follow-up, i.e. simultaneous measurements of fasting and 2-h glucose values during the OGTT, in addition to an HbA1c test, were selected for the present evaluation. Altogether, 122 women with complete glucose data attended the 1-year follow-up, 84 attended the 2-year follow-up, and 55 attended the 5-year follow-up. Since the incidence of type-2 diabetes is known to increase cumulatively within the first 1–5 years after GDM in pregnancy, we used the latest available set of complete glucose data from each woman for the present evaluation to ensure the longest possible follow-up time [9]. We also wanted to minimize the risk of selection bias by using data taken from the same woman on several occasions. Accordingly, the final evaluation was based on data from 55 women at 5-year follow-up, 48 women at 2-year follow-up, and 37 women at 1-year follow-up.

Of the 140 women who were included, 72 (51%) were of Nordic origin (all but two of them Swedish). Women of non-Nordic origin were immigrants from different countries in Southern and Eastern Europe, Asia, South America, and Africa, with Arab women form the Middle East (17%) and women from former Yugoslavia (10%) comprising the largest groups.

A standard 75-g OGTT was performed after overnight fasting. A Venflon catheter (Becton Dickinson, Helsingborg, Sweden) was inserted into an antecubital vein. Blood samples were drawn in duplicate at 0 and 120 min for determination of glucose concentrations, and the mean value was calculated. A blood sample for determination of HbA1c was collected in an EDTA-containing tube. Weight and height were recorded and body mass index (BMI) was calculated.

Based on the results of the OGTTs, four subgroups were defined according to the WHO (1999) criteria: (1) normal glucose tolerance, fasting blood glucose (FBG) <5.6 mmol/l, and 2-h blood glucose (2-h BG) <6.7 mmol/l; (2) impaired fasting glucose (IFG), FBG 5.6–6.0 mmol/l, and 2-h BG <6.7 mmol/l; (3) impaired glucose tolerance (IGT), FBG <6.1 mmol/l, and 2-h BG 6.7–9.9 mmol/l; and (4) diabetes mellitus, FBG ≥6.1 mmol/l, and/or 2-h BG ≥10 mmol/l [10]. Glucose homeostasis was also determined based on HbA1c levels according to the WHO and ADA recommendations; ≥ 6.5% (≥48 mmol/mol) suggesting diabetes; 5.7–6.4% (39–47 mmol/mol) suggesting high risk (pre-diabetes); and <5.7% (<39 mmol/mol) suggesting normal glucose homeostasis [1,3]. For comparison, the combined category "IFG and IGT" was used to represent pre-diabetes and the combined category "IFG, IGT, and diabetes" was used to represent abnormal glucose tolerance. Similarly, HbA1c levels of ≥5.7% (≥39 mmol/mol) were used to define abnormal glucose homeostasis.

Informed consent was obtained from all participants, and the study protocol was approved by the Ethics Committee of Lund University.

Assays

The HemoCue glucose system (HemoCue AB, Ängelholm, Sweden) was used for immediate measurement of whole blood glucose concentrations (in mmol/l). The mean coefficient of variation (CV) of the duplicate analyses performed in this study was 3.1% for fasting samples and 1.9% for 2-h samples. HbA1c was analyzed by ion-exchange chromatography, Mono S-HPLC [11]. The within-assay CV (on the Mono S scale) of this method is 0.47–0.94% and the between-assay CV is 1.68%. The Mono S method, together with the reference method from NGSP, is a designated comparison method in the IFCC (International Federation of Clinical Chemistry) Reference System [12]. Numbers given in % (Mono S) were converted to NGSP units (%) and IFCC units (mmol/mol) using the regression equations developed by the IFCC Working Group [12].

Statistical analysis

The agreement between diagnoses resulting from HbA1c and OGTT criteria was estimated by constructing cross tables. The κ coefficient (κ) was calculated, where the closer the value is to 1, the better the agreement [13]. Spearman's correlation was used to analyze the relationship between glucose and HbA1c values. A receiver operating characteristic (ROC) curve was constructed for HbA1c using OGTT as the gold standard for the diagnosis of abnormal glucose tolerance, and the area under the curve (AUC) was calculated. Diagnostic accuracy was assessed using sensitivity, specificity, positive predicted value (PPV), and negative predictive value (NPV).

Statistical analyses were performed with IBM SPSS Statistics 22 for Windows (IBM Corporation, New York, NY). Any p-value of less than 0.05 was considered statistically significant.

Results

Mean (±SD) values for age and BMI of the women included were 35.4 ± 5.6 years and 26.6 ± 2.3 kg/m^2, respectively. A median (interquartile range) of 26 (21–60) months had elapsed since their GDM pregnancy. Based on the OGTT, 62 women (44.3%) had normal glucose tolerance, 50 (35.7%) had pre-diabetes (13 IFG, 37 IGT), and 28 (20.0%) had diabetes. Among the 37 women with IGT, 12 had FBG values within the IFG range. In 8 women, the diagnosis of diabetes was based on the 2-h glucose value alone and in 6 women it was based on the fasting glucose value alone. In contrast, using the HbA1c criteria for definition, the corresponding figures for normal glucose homeostasis, pre-diabetes, and diabetes were 114 (81.4%), 21 (15.0%), and 5 (3.6%), respectively. In four of the five HbA1c tests that were consistent with a diagnosis of diabetes, the OGTT revealed diabetes, and in the remaining test it revealed IGT. The sensitivity of HbA1c for diabetes diagnosis was 14.3% and the

Table 1
Cross-tabulation between HbA1c, fasting blood glucose, and oral glucose tolerance test criteria in categorization of abnormal glucose metabolism

Test criteria	Normal OGTT	Abnormal OGTT
HbA1c ≥5.7% (≥39 mmol/mol)	3	23
HbA1c <5.7% (<39 mmol/mol)	59	55
FBG ≥5.6 mmol/l	0	49
FBG <5.6 mmol/l	62	29
HbA1c ≥5.7% (≥39 mmol/mol) or FBG ≥5.6 mmol/l	3	52
HbA1c <5.7% (<39 mmol/mol) and FBG <5.6 mmol/l	59	26

FBG, fasting blood glucose; OGTT, oral glucose tolerance test.

specificity was 99.1%. The agreement between HbA1c and OGTT in classifying diabetes or non-diabetes was poor, as indicated by a κ coefficient of 0.194.

Altogether 23 of 140 women (16.4%) met the combined criteria for abnormal glucose tolerance (both OGTT criteria and HbA1c criteria) (Table 1). The consistency in classifying abnormal glucose tolerance between HbA1c and OGTT criteria was 59% (82/140) and κ was 0.227, indicating poor agreement. Similar results were obtained when evaluating Nordic and non-Nordic women as separate groups (κ 0.278 and κ 0.166, respectively), or when evaluating the 1-, 2- and 5-year results separately (κ 0.260, κ 0.072 and κ 0.337, respectively). Combining HbA1c criteria with fasting glucose criteria improved the agreement for the total group to fair (79%, $\kappa = 0.596$), although it was no better than between FBG criterion alone and OGTT criteria (79%, $\kappa = 0.599$).

Correlations of HbA1c with FBG were 0.353 ($p < 0.001$) at 1- to 2-year follow-up and 0.613 ($p < 0.001$) at 5-year follow-up. The corresponding figures for HbA1c versus 2-h glucose were 0.380 ($p < 0.001$) and 0.430 ($p < 0.001$), respectively.

An ROC curve was constructed to evaluate the sensitivity and specificity of HbA1c in detection of abnormal glucose tolerance, as defined by the OGTT (Fig. 1). The optimal cut-off point of HbA1c for predicting abnormal glucose tolerance was 5.2% (33 mmol/mol) (AUC = 0.708, 95% CI 0.624–0.793), sensitivity was 69.2%, and specificity was 59.7%.

Table 2 shows the sensitivity, specificity, PPV, and NPV of HbA1c and FBG, or a combination of both diagnostic tests, relative to the OGTT (the gold standard) for various cut-offs. Overall, the FBG test alone showed better performance than the HbA1c test alone in detecting abnormal glucose tolerance. Of those who screened positive using the FBG test alone, all had (by definition) abnormal glucose tolerance (13 IFG, 12 IGT, 24 diabetes), as compared to 32% of those who screened negative (25 IGT, 4 diabetes). The combined use of HbA1c and FBG criteria showed performance similar to that with use of the FBG test alone.

We then tested a combination of FBG (≥5.6 mmol/l) with various cut-points of HbA1c to increase the sensitivity and NPV of the combined test. From this, HbA1c ≥5.0% (≥31 mmol/mol) was judged as an optimal cut-point, according to which, in addition to the 49 women who screened positive by FBG criterion alone, another 59 women were identified (38 with normal glucose tolerance, 17 with IGT, and four with diabetes by OGTT). Of the remaining 32 women who screened negative using this combination, 8 had abnormal glucose tolerance (all IGT) by OGTT.

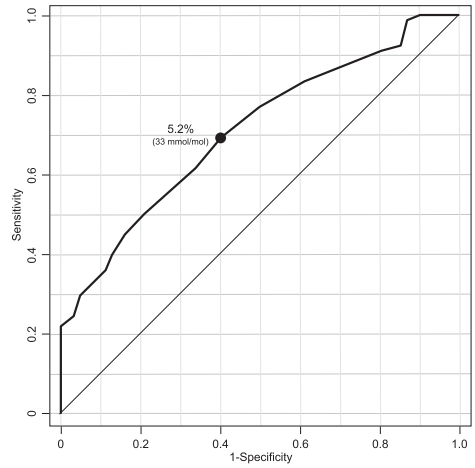

Figure. 1. Receiver operating characteristic curve for HbA1c for detection of abnormal glucose tolerance by the oral glucose tolerance test. The optimal cut-off point of HbA1c is indicated.

Discussion

In this historical cohort of women who had had GDM and who were prospectively followed for up to 5 years after delivery, we found suboptimal performance of proposed cut-points of HbA1c relative to OGTT in diagnosis of diabetes and abnormal glucose tolerance post-partum. Combined with a fasting glucose test, the diagnostic accuracy improved—although to an extent similar to that obtained using the fasting glucose test alone.

Women with a history of GDM have a 7.7-fold increased risk of future development of type-2 diabetes [4]. It has been shown previously that lifestyle intervention can prevent or delay the onset of type-2 diabetes in women with IGT and a history of GDM [14]. Thus, re-evaluation after pregnancy is essential. However, studies have repeatedly shown poor compliance with recommended guidelines in clinical practice, and the women fail to attend the post-partum visit, even in a research setting [15–18]. Easy, cost-effective, and

Table 2
Diagnostic indices of various criteria using HbA1c or fasting blood glucose to detect abnormal glucose tolerance

Diagnostic test	n[a]	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
HbA1c ≥5.7% (≥39 mmol/mol)	26	29.5	95.2	88.5	51.8
FBG ≥5.6 mmol/l	49	62.8	100.0	100.0	68.1
HbA1c ≥5.7% (≥39 mmol/mol) or FBG ≥5.6 mmol/l	55	66.7	95.2	94.5	69.4
HbA1c ≥6.0% (≥42 mmol/mol)	17	21.8	100.0	100.0	50.4
HbA1c ≥6.0% (≥42 mmol/mol) or FBG ≥5.6 mmol/l	51	65.4	100.0	100.0	69.7
HbA1c ≥5.0% (≥31 mmol/mol)	103	83.3	38.7	63.1	64.9
HbA1c ≥5.0% (≥31 mmol/mol) or FBG ≥5.6 mmol/l	108	89.7	38.7	64.8	75.0

FBG, fasting blood glucose; PPV, positive predictive value; NPV, negative predictive value.
[a] Number of women who met cut-off values.

less time-consuming screening strategies are required to capture as many women as possible who are at risk of type-2 diabetes. In this context, the HbA1c test appears to be attractive and its validity as a screening tool for abnormal glucose metabolism after GDM has only been examined in a few studies, with somewhat conflicting results [17,19–22].

Using the HbA1c test alone, we found that less than 5% of the women classified as having normal glucose tolerance by OGTT criteria would be misclassified as having abnormal glucose homeostasis, and more importantly, that 71% of the women classified as having abnormal glucose tolerance by OGTT criteria would be misclassified as having normal glucose homeostasis. Proposed cut-points of HbA1c had low sensitivity and modest NPV in detection of any degree of abnormal glucose tolerance, and therefore do not appear to be suitable for screening in these women. However, because of high PPV and high specificity, it may be used as a confirmatory test of the actual glucose tolerance status. The FBG test criterion had moderate sensitivity and NPV in detection of abnormal glucose tolerance. Megia et al. reported almost identical results to ours regarding HbA1c for diabetes diagnosis, with a sensitivity of 16.7% and a specificity of 100% [19]. However, using HbA1c of 5.7 (39 mmol/mol) as cut-off for any kind of impaired glucose tolerance, the sensitivity was comparatively low (13.5%). In contrast, Katreddy et al. reported a sensitivity of 71% and a specificity of 99% (AUC 0.98) in the diagnosis of diabetes, although the sensitivity of HbA1c ≥6.0% (≥42 mmol/mol) for detecting abnormal glucose tolerance was low (28%) [20]. Another study by Kim et al., based on a small group of women who had had GDM, found a sensitivity 65% and a specificity 68% for HbA1c ≥ 5.7% (≥39 mmol/mol) in detection of abnormal glucose tolerance [21]. ROC curves gave results similar to ours, with an AUC for any degree of impaired glucose tolerance of 0.76.

In line with previous studies, we found poor agreement in the consistency between HbA1c and OGTT criteria in classifying diabetes and abnormal glucose tolerance post-partum, although correlations between HbA1c and glucose values obtained during the OGTT indicated fairly good agreement [17,19,21].

Based on the present findings, the combination of HbA1c and FBG criteria classified 33% of the women who were classified as having abnormal glucose tolerance by OGTT criteria, as having normal glucose homeostasis. The specificity and PPV were high, but this combination did not improve the sensitivity and specificity obtained by FBG criterion alone. Similar observations for the combined test relative to the fasting glucose test alone were made by Picon et al. and Megia et al., albeit with higher sensitivities (83% and 82%, respectively), which might in turn be partly explained by their use of somewhat lower cut-offs [17,19]. Predictive values were only reported in the study by Picon et al., who found an NPV of 85%. Noctor et al. used a similar approach with cut-offs identical to those used by Picon et al., and reported sensitivity of 90% and NPV of 97% in detecting abnormal glucose tolerance, thereby reducing the proportion of women requiring confirmatory testing to 31%, as compared to 29% in the study by Megia et al. and 47% in the study by Picon et al. [22].

There are several plausible explanations for the discrepant results between studies. Firstly, differences in diagnostic criteria for the diagnosis of GDM imply that more or less high-risk women will be identified, rendering comparisons less reliable. If high glucose cut-points are used more severely affected women will be selected. We have recently evaluated how the introduction of the new International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria and the 1999 WHO criteria would affect the prevalence of GDM in our population [23]. The results indicate that 26% more women would be identified by the IADPSG criteria and 20% more women by the WHO criteria, compared with the modified EASD criteria presently in use. The prevalence of abnormal glucose tolerance post-partum observed in the present study was relatively high (55.7%) in comparison with other studies, ranging from 18.4% in the study by Noctor et al. to 68.5% in the study by Kim et al. This could partly be attributed to the different glucose cut-points used for the diagnosis of GDM. Differences in the interval to post-partum retesting between studies would also affect the results. Median interval to follow-up in the present study was 2.1 years as compared with 3 months (Megia et al.), 1 year (Picon et al.), 1.5 years (Kim et al.) and 2.6 years (Noctor et al.). Other important differences include patient characteristics, such as BMI and age, and the ethnic composition of the cohorts, which in turn may have an impact on the interpretation of HbA1c data *per se* [24]. Also, the HbA1c assays used may differ and may not be fully comparable.

The rationale for recommending OGTT post-partum in women with GDM is not only to detect women with apparent diabetes but also to identify women with pre-diabetes and IGT in whom diabetes can be delayed or prevented [14,25]. We therefore hypothesized that a reasonable screening model would be to accept all women with IFG for intensive follow-up and prevention without retesting—in our sample, corresponding to 35% of the study population (49/140). If we then accept HbA1c 5.0% (31 mmol/mol) as a cut-off for further identification, that would leave 59 women for confirmatory testing by OGTT, among whom 36% (21/59) would be diagnosed with diabetes or IGT based on the 2-h glucose value alone. Of the remaining 32 women, 25% would be misclassified as having normal glucose metabolism, i.e. 10% (8/78) of the women with any kind of abnormal glucose tolerance in the study cohort.

Several limitations of this study must be considered. Since it was based on historical data, the results may not be completely representative of the contemporary population. Furthermore, glucose concentrations were determined in whole blood, which was the routine in Sweden at the time of the study. Converted glucose thresholds provided by the WHO (1999) for whole blood were used for classification. Due to the higher water concentration in plasma than erythrocytes, glucose concentration in plasma is higher than glucose in whole blood. A constant factor of 1.11 for the conversion between concentration of glucose in blood and the equivalent concentration in plasma is recommended, assuming a normal hematocrit [26]. Since the concentration of glucose in whole blood depends on the hematocrit the WHO conversion tables may be inaccurate in some situations. We have no information on the hematocrit in these women, but they were all apparently healthy and we do not believe this should have any major impact on the results. One further limitation of the study is that each test was only performed once and the diagnosis of diabetes was not confirmed by a repeat test. Moreover, hemoglobinopathies were not systematically assessed but are generally more common in the Mediterranean and not-white populations. However, the agreement between HBA1c and OGTT results did not markedly improve by excluding non-Nordic women from the evaluation (κ 0.278 as compared with κ 0.227).

This is the first study in Sweden comparing the performance of HbA1c with that of established glucose criteria during the OGTT in women with previous GDM. The strengths of the study include the prospective design with long-term follow-up of a relatively large number of women after GDM pregnancy. The uniform diagnostic procedure for GDM used in southern Sweden since 1995 is also noteworthy: it is based on a universal 75-g OGTT and there have been no major changes. Furthermore, the HbA1c assay used has a known and constant relation to the IFCC standard [12].

In summary, proposed thresholds of HbA1c (≥6.5% [48 mmol/mol] and ≥5.7% [≥39 mmol/mol]) had low sensitivity in diagnosis of diabetes and abnormal glucose tolerance in the present study cohort. Combined with a fasting glucose test, the performance was no better than using a fasting glucose test alone. Considering that

early detection of pre-diabetes is of utmost importance in these women to prevent the development of diabetes, combining a fasting glucose test with a lower cut-point of HbA1c may be an alternative approach to select women for an OGTT—to identify those who have isolated post-glucose load hyperglycaemia. With an HbA1c cut-off of ≥5.0% (≥31 mmol/mol), the number of women who would need a confirmatory OGTT decreased by almost 60%, thus overlooking 10% of those with abnormal glucose tolerance in the study cohort.

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Paper III

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RESEARCH ARTICLE Open Access

The relative importance of maternal body mass index and glucose levels for prediction of large-for-gestational-age births

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Abstract

Background: The risk of gestational diabetes mellitus (GDM) increases substantially with increasing maternal body mass index (BMI). The aim of the present study was to evaluate the relative importance of maternal BMI and glucose levels in prediction of large-for-gestational-age (LGA) births.

Method: This observational cohort study was based on women giving birth in southern Sweden during the years 2003–2005. Information on 10 974 pregnancies was retrieved from a population-based perinatal register. A 75-g oral glucose tolerance test (OGTT) was performed in the 28 week of pregnancy for determination of the 2-h plasma glucose concentration. BMI was obtained during the first trimester. The dataset was divided into a development set and a validation set. Using the development set, multiple logistic regression analysis was used to identify maternal characteristics associated with LGA. The prediction of LGA was assessed by receiver-operating characteristic (ROC) curves, with LGA defined as birth weight > +2 standard deviations of the mean.

Results: In the final multivariable model including BMI, 2-h glucose level and maternal demographics, the factor most strongly associated with LGA was BMI (odds ratio 1.1, 95 % confidence interval [CI] 1.08–1.30). Based on the total dataset, the area under the ROC curve (AUC) of 2-h glucose level to predict LGA was 0.54 (95 % CI 0.48–0.60), indicating poor performance. Using the validation database, the AUC for the final multiple model was 0.69 (95 % CI 0.66–0.72), which was identical to the AUC retrieved from a model not including 2-h glucose (0.69, 95 % CI 0.66–0.72), and larger than from a model including 2-h glucose but not BMI (0.63, 95 % CI 0.60–0.67).

Conclusions: Both the 2-h glucose level of the OGTT and maternal BMI had a significant effect on the risk of LGA births, but the relative contribution was higher for BMI. The findings highlight the importance of concentrating on healthy body weight in pregnant women and closer monitoring of weight during pregnancy as a strategy for reducing the risk of excessive fetal growth.

Keywords: Body mass index, Gestational diabetes mellitus, Glucose levels, Large-for-gestational-age, Oral glucose tolerance test, Predicting risk

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Background

Obesity is an increasing health problem, and affects up to one-third of women of reproductive age in the western world [1]. The risk of gestational diabetes mellitus (GDM) increases substantially with increasing maternal body mass index (BMI) [2]. Moreover, GDM and maternal obesity are independently associated with adverse neonatal outcomes, in particular macrosomia and large-for-gestational-age (LGA) births [3–5], which in turn increase the risk of complications in both the mother and the newborn [6]. For the mother this includes prolonged labour, perineal lacerations, uterine atonia, abnormal haemorrhage and caesarean section [6, 7]. Neonatal complications consist of birth trauma associated with shoulder dystocia, hypoglycaemia, respiratory distress and may also result in impairment to health later in life [6, 7]. Antenatal detection of large fetuses makes it possible to intervene by induction of labour or caesarean section, thereby preventing the birth of macrosomic newborns or complications associated with vaginal delivery of large babies. Surkan et al. reported an unadjusted increase in LGA births in Sweden of 23 % over the years 1992–2001. The increasing trend could mainly be explained by concurrent increases in maternal BMI and decreases in maternal smoking [8]. The prevalence of maternal smoking has declined continuously in Sweden during the last decades with an annual change of 7.2 % between 2000 and 2008 [9].

Universal screening for GDM by an oral glucose tolerance test (OGTT) has been performed at the general antenatal clinics in southern Sweden since 1995. The screening program is well implemented and has previously shown high adherence, with 93 % of eligible women being screened [10]. During the years 2003–2005, pregnant women representing different glucose categories according to the 2-h glucose level of the OGTT were invited to take part in a follow-up program, the Mamma Study. The pregnancy outcomes of the participating women have been reported previously, indicating that even limited degrees of maternal hyperglycemia affect the outcome and increase the risk of LGA births [11]. During the period of recruitment to the Mamma Study, a large number of test results from the antenatal clinics were made available. These form the basis of the present study. The purpose was to evaluate the relative importance of BMI and glucose levels in prediction of LGA births in a large sample of the pregnant population, also taking other risk factors into account by adding information on maternal characteristics.

Methods

GDM screening

The screening program for GDM in southern Sweden has been described in detail previously [11]. Briefly, a 75-g OGTT is offered to all women in the 28 week of gestation, and is done after overnight fasting at their local antenatal clinic. The diagnostic criteria for GDM are a simplification of those recommended by the European Association for the Study of Diabetes, omitting the initial fasting glucose sample and defining GDM as a 2-h capillary blood glucose concentration of ≥ 9.0 mmol/L [12]. In 2004, routine glucose measurements in Sweden were switched from blood glucose measurements to plasma glucose measurements, and a transformation factor of 1.11 was agreed on [13], resulting in a 2-h threshold value of 10.0 mmol/L for capillary plasma glucose to define GDM. The HemoCue blood glucose system (HemoCue AB, Ängelholm, Sweden) is used to obtain immediate analysis of glucose concentrations. If 2-h capillary plasma glucose concentration is 8.9–9.9 mmol/L, indicating gestational impaired glucose tolerance (IGT), the OGTT is repeated within a week. Normal glucose tolerance during pregnancy is defined as a 2-h capillary plasma glucose concentration < 8.9 mmol/L.

Study population

Recruitment to the Mamma Study took place in 2003–2005, and involved four of the five delivery departments in the county of Skåne in southern Sweden; details have been described previously [11]. During the recruitment period, OGTT results from the local antenatal clinics were sent to the study coordinator (EA), enabling identification of the test results of women who consented to be enrolled; it also ensured correct sampling technique [10]. Initially, 11 976 OGTT results in total were reported. If a woman had repeated pregnancies during the period, only the first one was included. Likewise, if a repeat OGTT was performed, only the first one was included.

Participating women received standard obstetric care as long as their OGTT values were normal. Women diagnosed with GDM were transferred to specialist antenatal care and had regular contact with a diabetologist. They were given advice on diet and physical exercise, and they were closely monitored through self-testing of blood glucose. If treatment goals for blood glucose were not achieved, insulin treatment was added. Women diagnosed with gestational IGT were given advice on diet and physical exercise, but followed the routine pregnancy program, unless a repeat OGTT was diagnostic of GDM.

The study was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants and the study protocol was approved by the Ethics Committee of Lund University (LU 259–00).

Perinatal Revision South (PRS)

Population-based information was retrieved from the regional perinatal database, Perinatal Revision South (PRS),

which was established in 1995 for quality assurance in perinatal care in the southern region of Sweden [14]. The PRS is based on approximately 18 000 annual births, and is compiled from data reported by all delivery and neonatal units in the region. The maternal pregnancy characteristics used as exposure variables were maternal age at delivery, parity, BMI, maternal height and maternal smoking. Information about BMI (kg/m^2) was based on weight and height measured at the first prenatal visit in the first trimester. Gestational age was estimated from expected date of parturition according to ultrasound in the first half of gestation. LGA births, small-for-gestational-age (SGA) births and adequate-for-gestational-age (AGA) births were defined as birth weight greater than +2 standard deviations (SD), less than −2 SD and between −2 SD and +2 SD of the expected birth weight for gestational age and gender, respectively, according to the Swedish reference curve for fetal growth [15]. Of the 11 976 OGTT results, information in the PRS was available for a total of 11 016 pregnancies. When we evaluated the risk factors for LGA, infants with unavailable LGA information were excluded, and this restricted dataset was the basis of the present evaluation (n = 10 974). The dataset was divided into two parts, with every second woman belonging to the development dataset or the validation dataset.

Table 1 Maternal and infant characteristics according to glucose quartiles, and the corresponding 2-h plasma glucose level

Glucose quartiles (mmol/L)	<5.7		5.7–6.4		6.5–7.2		>7.20		2-h Glucose (mmol/L)		p^a
	n	%	n	%	n	%	n	%	mean	95 % CI	
Total	2637	23.9	2783	25.3	2819	25.6	2777	25.2			
Maternal age, years											<0.001
<20	80	32.5	62	25.2	63	25.6	41	16.7	6.2	6.1–6.4	
20–34	2148	24.2	2288	25.8	2264	25.5	2180	24.5	6.5	6.4–6.5	
≥35	409	21.6	433	22.9	492	26.0	556	29.4	6.6	6.6–6.7	
Parity											0.09
1	128	23.8	134	24.9	141	26.2	135	25.1	6.5	6.4–6.5	
2–3	119	24.1	128	26.0	124	25.2	122	24.7	6.5	6.4–6.5	
≥4	16	24.1	15	22.5	15	23.4	20	30.0	6.6	6.5–6.7	
Smoker											<0.001
No	2220	23.4	2408	25.4	2430	25.6	2424	25.6	6.5	6.5–6.5	
Yes	341	27.2	309	24.6	333	26.6	271	21.6	6.3	6.3–6.4	
Maternal BMI, kg/m^2											<0.001
<18.5	50	25.6	50	25.6	50	25.6	45	23.1	6.4	6.3–6.6	
18.5–24	1496	25.1	1569	26.3	1542	25.9	1351	22.7	6.4	6.4–6.4	
25.0–29.9	585	22.0	641	24.1	687	25.9	743	28.0	6.6	6.5–6.6	
30–34.9	182	20.8	187	21.4	223	25.5	281	32.2	6.6	6.6–6.7	
≥35	83	20.1	103	25.0	93	22.6	133	32.3	6.8	6.7–6.9	
Gestational age, weeks											0.006
<37	117	20.0	148	25.3	153	26.2	167	28.5	6.7	6.5–6.8	
37–41 + 6	2345	24.0	2472	25.3	2502	25.6	2452	25.1	6.5	6.4–6.5	
≥42 + 0	175	26.5	163	24.7	164	24.8	158	23.9	6.4	6.3–6.5	
Weight for gestational age											<0.001
SGA	69	23.2	80	26.9	68	22.9	80	26.9	6.5	6.4–6.7	
AGA	2446	24.2	2577	25.5	2578	25.6	2495	24.7	6.5	6.4–6.5	
LGA	115	20.1	110	19.2	156	27.3	191	33.4	6.7	6.6–6.9	
Infant gender											0.9
Male	1407	24.5	1415	24.5	1437	25.0	1479	25.8	6.5	6.4–6.5	
Female	1228	23.4	1359	25.8	1379	26.2	1292	24.6	6.5	6.5–6.5	

BMI body mass index, *CI* confidence interval, *SGA* small-for-gestational-age, *AGA* adequate-for-gestational-age, *LGA* large-for-gestational-age
[a]*p*-values obtained by non-parametric tests (Kruskal-Wallis) for difference in glucose level between the specified groups

Statistical analysis

Differences in glucose levels between groups were assessed using the Kruskal-Wallis test.

Chi-squared tests were performed to test possible differences between the datasets regarding maternal and infant characteristics (i.e. the development dataset and the validation dataset). The correlation between maternal BMI and 2-h glucose levels was estimated using the Pearson rho, and the linear relationship was estimated using a simple linear regression.

The prediction model for LGA was developed on the development dataset using univariate and multivariable logistic regression analyses. The variables tested were: maternal age (in years; continuous variable), parity 1, parity ≥ 4 (with parity 2–3 as reference), maternal smoking (yes/no), maternal BMI (in kg/m^2; continuous), maternal height (in cm; continuous), and glucose levels (in mmol/L; continuous). Models including class variables or second-degree polynomials were tested, but were abandoned as they performed worse than the models including the linear, continuous variables mentioned. Variables with a crude p-value of < 0.05 in their association with LGA in the univariate model were entered into a multiple model, and variables with a p-value of < 0.05 in the multiple model were entered into the final multiple model. A two-sided p-value of less than 0.05 was considered statistically significant.

The results obtained from the final multiple model, and two other models for comparison, were applied to the validation dataset. The performance of each model was evaluated by studying the area under the receiver-operating characteristics (ROC) curve (AUC). The variance of each AUC was computed using the method proposed by DeLong et al. [16].

All statistical analyses were performed using Gauss (Gauss™; Aptec Systems Inc., Maple Valley, WA, USA; http://www.aptech.com).

Results

The frequency of maternal and infant characteristics according to glucose quartile and the corresponding mean 2-h plasma glucose levels are given in Table 1. Of the 2777 women with glucose levels in the upper quartile, 120 (1.1 % of all women) fulfilled the glucose threshold for GDM (2-h plasma glucose concentration ≥ 10.0 mmol/L) and 301 (2.7 % of all women) fulfilled the glucose threshold for gestational IGT (2-h plasma glucose concentration 8.9–9.9 mmol/L). A linear regression analysis showed a weak, albeit statistically significant, linear association between maternal BMI and glucose levels (increase of 2-h plasma glucose per each BMI-unit: 0.022; 95 % CI 0.017–0.028), with a statistically significant, but weak correlation coefficient (Pearson rho: 0.074; 95 % CI: 0.056–0.093). A ROC curve based on the total dataset revealed that the ability of the 2-h glucose levels to predict LGA births was poor; AUC was 0.54 (95 % CI 0.48–0.60) (Fig. 1). Furthermore, there was no apparent natural cutoff point above which there would be an increased risk of LGA in the infant.

The maternal and infant characteristics of the development and validation groups are given in Table 2. The demographic characteristics of the groups were similar, but by chance there were significantly more women with BMI above 35, and SGA infants, in the development dataset than in the validation dataset.

Table 3 shows the odds ratios for LGA obtained from univariate and multiple logistic regression analyses based on the development sample. In the univariate analysis, all the factors evaluated except height ($p = 0.0831$, not shown) and parity ≥ 4 were significantly associated with LGA. In the first multiple model (including all the significant variables), all variables except maternal age remained significant. In the final multiple model, excluding maternal age, the factor most strongly associated with LGA was BMI ($p = 2.6 \times 10^{-19}$), accounting for 4.3 % of the variance in the univariate setting ($R^2 = 0.043$). Using the validation database, the AUC for the final multiple model was 0.69 (95 % CI 0.66–0.72), which was identical to the AUC retrieved from a model not including 2-h glucose (AUC 0.69 [95 % CI 0.66–0.72]), and larger than from a model including 2-h glucose but not BMI (AUC 0.63 [95 % CI 0.60–0.67]).

The overall abilities of the three models developed in predicting LGA in the validation sample were illustrated

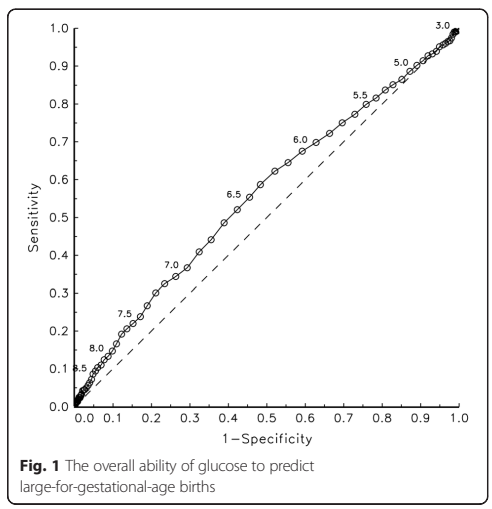

Fig. 1 The overall ability of glucose to predict large-for-gestational-age births

Table 2 Demographic characteristics of development sample and validation sample groups

Characteristic	Development sample (n = 5487)		Validation sample (n = 5487)		p^a
Maternal age, years	29.7	5.1	29.6	5.1	0.88
< 20	121	(2.2)	125	(2.3)	0.80
20–34	4415	(80.5)	4426	(80.7)	0.79
≥ 35	951	(17.3)	936	(17.1)	0.71
Parity					
1	2688	(49.0)	2681	(48.9)	0.90
2–3	2463	(44.9)	2465	(44.9)	0.97
≥ 4	336	(6.1)	341	(6.2)	0.84
Smoker					
No	4727	(86.1)	4722	(86.1)	0.89
Yes	625	(11.4)	623	(11.4)	0.96
Maternal BMI, kg/m^2	24.9	4.5	24.7	4.3	0.089
< 18.5	102	(1.9)	92	(1.7)	0.47
18.5–24	2928	(53.4)	3015	(54.9)	0.095
25.0–29.9	1303	(23.7)	1343	(24.5)	0.37
30–34.9	440	(8.0)	424	(7.7)	0.57
≥ 35	236	(4.3)	175	(3.2)	0.002
Gestational age, weeks	39.7	1.7	39.7	1.7	0.62
< 37	304	(5.5)	281	(5.1)	0.33
37–41 + 6	4875	(88.8)	4889	(89.1)	0.67
≥ 42 + 0	308	(5.6)	317	(5.8)	0.71
Weight for gestational age					
SGA	166	(3.0)	131	(2.4)	0.04
AGA	5044	(91.9)	5061	(92.2)	0.58
LGA	277	(5.0)	295	(5.4)	0.44
Infant gender					
Male	2839	(51.7)	2888	(52.6)	0.35
Female	2648	(48.3)	2599	(47.4)	0.35

Both groups contain only information where all information was available. Data are n (%) or mean (SD)
AGA adequate for gestational age, *BMI* body mass index, *LGA* large-for-gestational-age, *SGA* small-for-gestational-age
[a]*p*-values obtained by chi-squared test (1 DF) for class variables and by Mann-Whitney *U*-test for continuous data

Table 3 Risk factors for large-for-gestational-age infants in development sample, using univariate and multiple logistic regression analysis

	Univariate model		Multiple model		Final multiple model		
Risk factor	OR	*p*	OR	*p*	OR	95 % CI	*p*
Maternal age (per 1-year increase)	1.04	0.005	1.01	0.677			
Body mass index (per 1-step increase)	1.11	<0.001	1.10	<0.001	1.10	1.08–1.13	<0.001
2-h glucose (per 1 mmol increase)	1.12	0.003	1.09	0.033	1.09	1.01–1.18	0.028
Smoker	0.31	<0.001	0.29	<0.001	0.29	0.16–0.52	<0.001
Parity 1	0.48	<0.001	0.52	<0.001	0.51	0.40–0,67	<0.001
Parity ≥ 4	0.98	0.917					

Multiple model included variables with $p < 0.05$ in univariate model. Final multiple model included variables with $p < 0.05$ in primary multiple model
OR odds ratio, *CI* confidence interval

using ROC curves (Fig. 2). The figure clearly shows that the ROC curve based on the model including BMI, nulliparity and maternal smoking was identical to that based on the model in which glucose levels were also added, whereas the performance of the model that included glucose levels but not BMI was considerably poorer.

Discussion

The main findings of the present study were that both the 2-h glucose level of the OGTT and maternal BMI had a significant effect on the risk of delivering an LGA neonate. However, the relative contribution was much higher for BMI, even when taking other risk factors into account. The overall ability of the developed model to predict LGA in the validation sample was satisfactory, but was identical to that of a model that did not include the 2-h glucose level.

The lack of internationally uniform diagnostic criteria for GDM, and the lack of agreement regarding what glucose levels should define normal glucose tolerance during pregnancy, hampers comparisons between studies [17]. Similar to our study, using the 2-h threshold of the WHO 1999 criteria to define normal glucose tolerance during pregnancy [18], a Danish study investigated the relationship between pregnancy outcome and pregnancy overweight or obesity in 2459 women with normal glucose tolerance during pregnancy [19]. After adjustment for various risk indicators, including the 2-h glucose value during the OGTT, they found a progressively increased risk of LGA births in overweight and obese women. However, they did not evaluate the corresponding effect of glucose levels when controlling for BMI and other risk indicators. It should be noted that the LGA was defined as birth weight above the ninetieth percentile for the reference population, which differed from the one used in the current study (approximately equivalent to the 97.5th percentile).

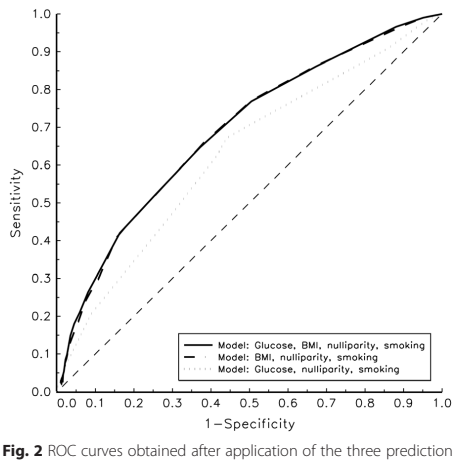

Fig. 2 ROC curves obtained after application of the three prediction models based on the validation data

Based on the ROC curve of the total dataset, we found no apparent natural cutoff point above which there would be an increased risk of having an LGA infant. This is in line with the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) Study, which showed that maternal hyperglycemia is associated with perinatal risk in a linear way, with no obvious threshold [20]. In a post hoc analysis using the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria for GDM [21], OR for birth weight greater than the ninetieth percentile was somewhat higher in non-obese GDM women (2.19, 95 % CI 1.93–2.47) than in obese non-GDM women (1.73, 95 % CI 1.50–2.0) relative to non-obese non-GDM women, controlling for other potential risk factors [4]. Whereas all other guidelines for the diagnosis of GDM are more or less based on arbitrary statistics, the IADPSG criteria are for the first time based on perinatal outcomes [22]. According to these criteria, at least one of the fasting, 1-h or 2-h venous plasma glucose thresholds during a 75-g OGTT (5.1, 10.0 or 8.5 mmol/L, respectively) must be equalled or exceeded to make a GDM diagnosis. Use of the individual glucose thresholds fasting, 1-h and 2 h identified 55, 55 and 38 %, respectively, of the total HAPO cohort [23]. Although it is not regarded as a diagnostic standard [21], capillary glucose samples are widely used for diagnostic purposes in Sweden. According to a recently presented conversion algorithm, the capillary 2-h threshold value of 10.0 mmol/L—used in most parts of Sweden to define GDM [24] —coincides with the venous 2-h threshold value proposed by the IADPSG [25]. From this, it is obvious that the simplified method, omitting the initial fasting glucose sample during the OGTT, is not optimal for prediction of gestational weight of the newborn.

The main strength of the present study was the uniform diagnostic procedure for GDM, based on universal screening with a 75-g OGTT, enabling identification of a rather large cohort of women with test results over the entire glucose scale. In our previous report from the Mamma Study, suggesting that moderately increased glucose levels may also affect pregnancy outcome, adjustments for BMI were not performed because the information was not available at the time [11]. In light of the present findings, it is reasonable to assume that adjustment for BMI would have attenuated the results to some extent. However, as the current study showed that the correlation between BMI and glucose levels was rather weak, it is not likely that the results would be

heavily influenced from BMI. Furthermore, since the control group in the previous study included only one twenty-fourth of consenting women with normal glucose tolerance during pregnancy, the material did not allow prediction analysis. We have previously shown that maternal characteristics such as age, parity and smoking—in addition to BMI and maternal glucose status—influence fetal growth during the last trimester [26]. The logistic regression modelling identified the independent variables available from the register that are important and can help in the prediction of LGA births.

It could be argued that women with glucose levels in the IGT range and above, receiving some kind of advice or treatment during pregnancy may have biased the results. However, it is likely that the risk of LGA births would have increased even more if these women had not been taken care of. Another possible weakness of the study was the lack of information regarding ethnicity. Disparities in ethnicity/race may affect the impact of obesity and glucose status on perinatal outcomes [27–29]. Furthermore, the prediction model might have been more powerful if maternal weight gain during pregnancy had been considered. Both maternal pre-pregnancy obesity and excessive gestational weight gain lead to increased risk of adverse pregnancy outcomes, including LGA. Overall, the associations between maternal pre-pregnancy obesity and adverse pregnancy outcomes appear to be stronger than those between excessive gestational weight gain and adverse pregnancy outcomes [30], although some studies have indicated that gestational weight gain is of greater importance [5, 31].

Conclusions

Based on the present material, we conclude that maternal BMI had a greater impact on the prediction of LGA birth than the 2-h glucose level of the OGTT. The overall performance of the full prediction model, also taking other risk factors into account, was satisfactory. The data highlight the importance of targeting healthy body weight in pregnant women and closer monitoring of weight during pregnancy as a strategy for reducing the risk of excessive fetal growth. A number of intervention trials have been published and show heterogeneous results in efficacy in reducing excess gestational weight gain [32, 33]. Adequately powered intervention studies are needed to provide evidence-based guidelines to facilitate pregnant women in achieving weight gain within recommended limits with the aim to reduce neonatal adiposity.

Abbreviations

AGA: adequate-for-gestational-age; AUC: area under the ROC curve; BMI: body mass index; CI: confidence interval; GDM: gestational diabetes mellitus; HAPO: Hyperglycemia and Adverse Pregnancy Outcome; IADPSG: International Association of Diabetes and Pregnancy Study Groups; IGT: impaired glucose tolerance; LGA: large-for-gestational-age; OGTT: oral glucose tolerance test; OR: odds ratio; PRS: Perinatal Revision South; ROC curve: receiver-operating characteristic curve; SD: standard deviation; SGA: small-for-gestational-age; WHO: World Health Organization.

Competing interests

The authors declare that they have no competing interests.

Authors'contributions

KB contributed to the conceptual design, acquired and interpreted the data, and drafted the article. EA contributed to the conceptual design, acquired and interpreted data and critically revised the manuscript. RC contributed to the conceptual design, interpreted the data and helped draft the article. CI contributed to the conceptual design, interpreted data and critically revised the manuscript. KK conceived and designed the study, analyzed and interpreted the data, and critically revised the manuscript. All the authors approved the final version and they vouch for the accuracy of the manuscript according to the guidelines given.

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Paper IV

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Original research

HbA1c as a predictor of diabetes after gestational diabetes mellitus

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ABSTRACT

Aim: We wanted to investigate third-trimester HbA1c as a predictor of diabetes after gestational diabetes mellitus (GDM).

Methods: Women with GDM were followed up prospectively for five years from pregnancy to detect the development of diabetes. The ability of HbA1c to predict diabetes was evaluated with receiver-operating characteristic (ROC) curves and logistic regression analysis.

Results: By five years, 73 of 196 women had been diagnosed with diabetes. An optimal cut-off point for HbA1c of 36 mmol/mol (5.4%) could predict diabetes with 45% sensitivity and 92% specificity. For HbA1c ≥39 mmol/mol (≥5.7%), sensitivity, specificity, and positive predictive value were 30%, 97%, and 91%, respectively. In logistic regression analysis, adjusting for the diagnostic glucose concentration during pregnancy, HbA1c levels in the upper quartile (≥36 mmol/mol) were associated with a 5.5-fold increased risk of diabetes.

Conclusion: Third-trimester HbA1c levels in the pre-diabetes range revealed women with post-partum diabetes with high specificity and high positive predictive value. HbA1c testing could be used as a strategy to select high-risk women for lifestyle interventions aimed at prevention of diabetes starting during pregnancy. The results should encourage further validation in other populations using new diagnostic criteria for GDM.

Abbreviations: AUC, area under the curve; BMI, body mass index; CI, confidence interval; EASD, European Association for the Study of Diabetes; GDM, gestational diabetes mellitus; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; NPV, negative predictive value; OGTT, oral glucose tolerance test; OR, odds ratio; PPV, positive predictive value; ROC, receiver-operating characteristic; SD, standard deviation; WHO, World Health Organization.

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1. Introduction

Post-partum follow-up of pregnancies with gestational diabetes mellitus (GDM) is important, as these women have a several-fold increased risk of progression to type-2 diabetes after delivery [1]. It has been shown that there is a beneficial effect of lifestyle intervention on the 10-year risk of diabetes in women with a history of GDM [2]. However, the uptake of post-partum screening after GDM is suboptimal, and women fail to attend the post-partum visit, even in a research setting [3–6]. An easy way of identifying those who are at highest risk of developing diabetes after pregnancy is needed, so that midwives and physicians can pay more attention to these women and start intervention already in pregnancy when the women are more likely to be highly motivated.

HbA1c analysis was recently endorsed as a screening test for unrecognized diabetes in early pregnancy [7–9], but it has not yet been advocated as a diagnostic test for GDM. There is some interest in finding an HbA1c threshold at other stages of pregnancy that could even be used for intervention during pregnancy. HbA1c as a diagnostic test has advantages for both patients and physicians. It can be performed without fasting, and is more reproducible and less cumbersome than an oral glucose tolerance test (OGTT) [10]. However, we and others have found a low sensitivity of HbA1c testing relative to an OGTT in diagnosing diabetes and pre-diabetes in women who have previously had GDM [5,11–16]. Very few studies have evaluated the clinical usefulness of third-trimester HbA1c levels as a way of predicting the development of post-partum diabetes [17–21]. In a previous study from our geographical area, we found that 30% of the women with GDM in the study cohort had already developed diabetes five years after delivery, and that HbA1c levels ≥38 mmol/mol (≥5.6%) at the diagnostic OGTT during pregnancy, corresponding to the upper quartile, were associated with a four-fold increased risk of developing diabetes [17]. The aim of the present study was to investigate the HbA1c level measured close to the twenty-eighth week of pregnancy as a predictor of diabetes development up to five years after pregnancy.

2. Material and methods

2.1. Participants

The prospective Mamma Study followed women in southern Sweden who gave birth during the years 2003–2005, for up to 5 years from delivery, to detect the development of post-partum diabetes. A detailed description of the study design has already been reported [6]. Briefly, pregnant women, representing different glucose categories according to an OGTT, were invited to take part in the study. A 75-g OGTT was offered to all women in the twenty-eighth week of gestation, excluding those who were diagnosed with diabetes before pregnancy. The diagnostic criteria for GDM were a slight modification of the European Association for the Study of Diabetes (EASD) criteria, defining GDM as a 2-h capillary blood glucose concentration of ≥9.0 mmol/l [22], corresponding to a plasma glucose concentration of ≥10.0 mmol/l [23]. Based on this definition, 391 women were recruited. HbA1c was measured within two weeks of the diagnosis of GDM. Participants were followed for the development of diabetes by means of an OGTT at 1–2 years and at 5 years after pregnancy—or until the diagnosis of diabetes. Based on the stated country of origin of at least three grandparents, women were grouped according to whether they were of European or non-European origin. Diagnostic criteria during follow-up were those proposed by the World Health Organization (WHO) 1999 [24]. According to the results of the OGTT, women were classified as having normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or diabetes. Participants gave written informed consent and the Ethics Committee of Lund University approved the study (LU 259-00), which was performed according to the Declaration of Helsinki.

2.2. Metabolic measurements

The HemoCue Glucose 201+ system (HemoCue AB, Ängelholm, Sweden) was used for immediate measurement of plasma glucose concentrations (mmol/l). HbA1c was measured with ion-exchange chromatography procedures (Variant II from BioRad; Tosoh G7 from Tosoh Bioscience; and in-house Mono S) with results that were traceable to the Mono S procedure at the Swedish Reference Laboratory. Values given in % (Mono S) were converted to NGSP units (%) and IFCC units (mmol/mol) using the regression equations developed by the IFCC Working Group [25].

2.3. Statistical analysis

HbA1c values are given as mmol/mol with % NGSP units in parentheses or brackets. Continuous variables are summarized by means and standard deviations (SDs) or 95% confidence intervals (CIs). Differences between group means were compared with analysis of variance (ANOVA). Logistic regression analysis was used to calculate the odds ratios (ORs) and 95% CI for 5-year diabetes risk in different quartiles of HbA1c levels. A receiver-operating characteristic (ROC) curve was plotted to evaluate the diagnostic performance of HbA1c in diabetes prediction. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the area under the curve (AUC) were calculated. Threshold for discrimination was calculated with the Youden index [26]. IBM SPSS Statistics 22.0 for Windows (IBM Corporation, Armonk, NY, USA) was used for analysis. Two-sided *p*-values of less than 0.05 were considered to be statistically significant.

3. Results

Of the 391 women who agreed to participate prospectively, 5-year data were available for 196 of them. Among these, 73% were of European origin (mostly Swedish) and 27% were of non-European origin (with Arab and Asian origin being the largest groups).

Mean values for maternal age, diagnostic 2-h plasma glucose concentration, and HbA1c level during pregnancy in participants were 33.3 (SD 4.9) years, 11.1 (1.7) mmol/l, and 33.1 (7.1) mmol/mol [5.2% (1.1%)], respectively. The correspond-

HbA1c cut-off	n[a]	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
≥48 mmol/mol (≥6.5%)	10	13.7	100.0	100.0	48.8
≥45 mmol/mol (≥6.3%)	12	16.4	100.0	100.0	49.6
≥42 mmol/mol (≥6.0%)	15	19.2	98.3	93.3	50.0
≥39 mmol/mol (≥5.7%)	24	30.1	96.7	91.2	53.2
≥36 mmol/mol (≥5.4%)	38	45.2	91.7	86.8	57.8
≥32 mmol/mol (≥5.1%)	75	71.2	61.7	69.3	63.8

PPV, positive predictive value; NPV, negative predictive value.
[a] Number of women who reached the threshold value.

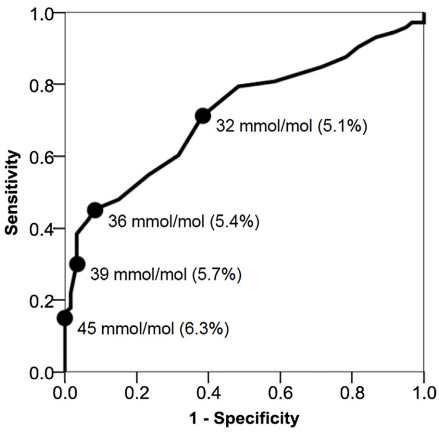

Fig. 1 – Predictive accuracy of HbA1c in detecting diabetes five years after gestational diabetes, using women with normal glucose tolerance as a reference. Various cut-off points are shown.

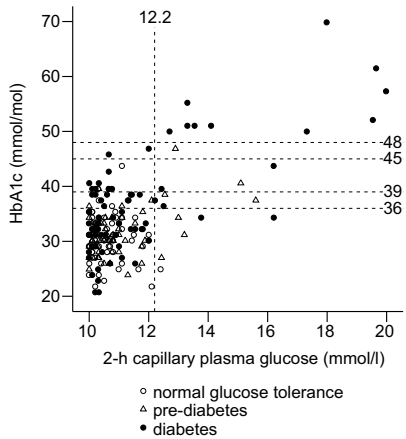

Fig. 2 – HbA1c levels plotted against the diagnostic 2-h glucose concentration during pregnancy for 196 women with gestational diabetes. Various diagnostic cut-off levels are shown, and the diagnoses at the 5-year follow-up are indicated by symbols.

ing figures for non-participants were 32.4 (5.8) years, 11.0 (1.1) mmol/l, and 32.7 (5.8) mmol/mol [5.1% (0.9%)], and the differences compared to participants were not significant. After five years, 73 women had been diagnosed with diabetes: 14 before the first follow-up, 25 at the first (1- to 2-year) follow-up, 13 between the first follow-up and the final (5-year) follow-up, and 21 at the final follow-up. Of the remaining 123 women who participated in the 5-year follow-up (out of a total of 144), 60 were classified as having NGT and 63 were classified as having IFG/IGT (pre-diabetes).

The mean HbA1c level during pregnancy in women who had developed diabetes after 5 years was 36.7 (95% CI: 34.5–38.8) mmol/mol [5.5% (5.3–5.7%)], as compared to 31.4 (30.4–32.4) mmol/mol [5.0% (4.9–5.1%)] in women with pre-diabetes and 30.6 (29.5–31.7) mmol/mol [4.9% (4.8–5.1%)] in women with NGT at 5 years ($p < 0.0001$).

Using NGT at 5-year follow-up as a reference, an ROC curve was constructed to evaluate HbA1c as a predictor of diabetes up to five years after pregnancy (Fig. 1). The ability of the ROC curve to predict diabetes was fair (AUC = 0.720, 95% CI: 0.634–0.806, $p < 0.0001$), with an optimal cut-off point of 36 mmol/mol (5.4%), resulting in a sensitivity of 45% and a specificity of 92%. Table 1 shows the sensitivity, specificity, PPV, and NPV for various cut-offs. Overall, HbA1c showed high specificity and PPV, but the sensitivity was low. The prediction did not improve by using both NGT and IFG/IGT at 5-year follow-up as a reference (AUC = 0.710, 95% CI: 0.630–0.791, $p < 0.0001$). Similar results were obtained when we included women of Nordic origin only (diabetes, n = 23 vs. NGT, n = 44; AUC = 0.734, 95% CI: 0.588–0.879, $p = 0.002$).

In Fig. 2, HbA1c levels are plotted against the diagnostic 2-h capillary plasma glucose concentrations during pregnancy for the whole study group. After five years, all ten women with HbA1c levels ≥48 mmol/mol (≥6.5%) had been diagnosed with diabetes, six women before the first follow-up (HbA1c 51–70 mmol/mol [6.8–8.6%]), one woman at the first follow-up (HbA1c 57 mmol/mol [7.4%]), and three women at the five-year follow-up (HbA1c 50–55 mmol/mol [6.7–7.2%]). Similarly, in 13 women with HbA1c levels ≥45 mmol/mol (≥6.3%) all but 1 woman (IGT) had been diagnosed with diabetes after five years. Altogether, five out of 27 women with HbA1c levels ≥39 mmol/mol (≥5.7%) had not been diagnosed with diabetes

after five years (2 NGT, 1 IFG, and 2 IGT). The corresponding figure for women with 2-h capillary plasma glucose levels ≥12.2 mmol/l (the diagnostic limit for diabetes outside of pregnancy) was eight out of 24 (1 NGT, 3 IFG, and 4 IGT).

HbA1c levels for the total study group were grouped into quartiles. Median levels for HbA1c in mmol/mol [%] in the respective quartiles were: 27 (range: 21–29) [4.6% (range: 4.1–4.8%)] (n = 56), 31 (30–31) [5.0% (4.9–5.0%)] (n = 43), 33 (32–35) [5.2% (5.1–5.4%)] (n = 51), and 40 (36–70) [5.8% (5.4–8.6%)] (n = 46). A logistic regression analysis, testing the predictive value of HbA1c quartiles for the 5-year diabetes risk, showed that women with HbA1c levels in quartile four had a seven-fold increased risk of post-partum diabetes compared to women with HbA1c levels in quartiles 1–3 (OR = 7.0, 95% CI: 3.3–14.6, $p < 0.0001$). This association remained significant after adjustment for maternal age and the 2-h glucose level during pregnancy (OR = 5.5, 95% CI: 2.5–12.1, $p < 0.0001$).

4. Discussion

The results of the present study confirm our previous findings that HbA1c levels in the upper quartile, measured close to the diagnostic OGTT during pregnancy, predict diabetes development during the five years after delivery [17]. To the best of our knowledge, only four other studies have investigated an association between HbA1c levels during pregnancy and the risk of post-partum diabetes [18–21].

Using the WHO (1999) criteria for the diagnosis of GDM, Liu et al. evaluated HbA1c, measured at 26–30 gestational weeks, as a predictor of diabetes 1–5 years after delivery in 1263 Chinese women [18]. Adjusting for various risk factors in a Cox proportional hazards model, the hazard ratio for post-partum diabetes was 2.11 (95% CI: 1.50–2.97) for every unit (%) increase in HbA1c. Furthermore, when fasting glucose, 2-h glucose, and HbA1c level during pregnancy were entered into the model simultaneously, 2-h glucose and HbA1c level, but not fasting glucose, remained significant and positive predictors of post-partum diabetes. In our previous study, both HbA1c and the fasting glucose level during pregnancy were found to be independent predictors of the 5-year diabetes risk [17]. A number of risk factors for diabetes development after GDM have been identified, which may in part differ from one population to another [27]. The fact that the fasting glucose levels did not predict diabetes in the study by Hsu et al. may be specific to the Chinese population, as fasting plasma glucose has been reported to be less sensitive for diagnosis of diabetes than the 2-h glucose level in the Asian population [28].

In a retrospective study from Korea, evaluating HbA1c at 26–30 gestational weeks as a diagnostic test for GDM, follow-up data for at least 3 months after pregnancy were available for 54 of 321 women [19]. Based on ROC-curve analysis, an optimal cut-off value for HbA1c of 37 mmol/mol (5.5%) could predict diabetes with 79% sensitivity and 73% specificity. However, the restricted number of women included in the analysis made the results less reliable. Furthermore, long-term follow-up data were not available. In another study from Warsaw, Poland, Malinowska-Polubiec et al. evaluated various risk factors for diabetes 0.5–10 years after pregnancy in 150 women with a history of GDM [20]. In that population both second-trimester and third-trimester HbA1c were associated with increased relative risks of post-partum diabetes. Finally, in a retrospective study of 305 women in the Czech Republic, Bartakova et al. found an optimal cut-off value from ROC-curve analysis (based on Youden statistics) for mid-trimester HbA1c of 36 mmol/mol (5.4%) for any degree of post-partum glucose abnormality during the first year after pregnancy [21].

In addition to the 2-h plasma glucose concentration during pregnancy, we have recently reported that (1) BMI at the first follow-up after pregnancy and (2) a non-European background were the most important risk factors for development of diabetes five years after pregnancy in the total Mamma Study cohort (defining GDM by the WHO (1999) criteria) [29]. However, HbA1c was not included in the prediction model since it was only measured in women diagnosed with GDM according to clinical routine (EASD criteria). For this reason, we performed a separate study restricted to these women.

At the time of the design of the Mamma Study, HbA1c was not recommended as a diagnostic test for diabetes, nor as a test early in pregnancy to rule out pre-existing diabetes. In our material, an HbA1c level of ≥48 mmol/mol (≥6.5%) during the third trimester of pregnancy identified all women with a diabetes diagnosis five years after pregnancy, some of whom had been diagnosed with diabetes before the first follow-up and may have had pre-gestational diabetes. Furthermore, an HbA1c level of ≥45 mmol/mol (≥6.3%) identified all but 1 woman with diabetes after five years, and an HbA1c level ≥39 mmol/mol (≥5.7%) identified all but 5 women with a diabetes diagnosis during follow-up. On the other hand, for the various thresholds, HbA1c had low sensitivity in diagnosing diabetes using either NGT or NGT/IFT/IGT as a reference. These data provide evidence to suggest there may be a useful HbA1c threshold above which all women should be closely monitored, starting already during pregnancy, to prevent diabetes development after delivery. Furthermore, after adjustment for the 2-h glucose level, HbA1c levels equal to and above the optimal cut-off level of the ROC curve were associated with more than a 5-fold increased risk of post-partum diabetes. This indicates that HbA1c analysis could be an adjunct to the OGTT in identifying women who are at high risk of developing post-partum diabetes.

A major limitation of the study was that HbA1c measurements were only available for women with a clinical diagnosis of GDM. Moreover, as previously reported [6], the overall participation rate in the Mamma Study was less than 50%, which may have introduced selection bias. As the diagnostic criteria for GDM have changed worldwide since the time when this study was initiated, the findings should be repeated and validated in future studies using updated diagnostic criteria [7–9]. Generalization to populations with different ethnic backgrounds should also be done. The strengths of the study were the uniform screening procedure in the region, based on a universally applied OGTT, and the prospective study design with long-term follow-up after pregnancy.

5. Conclusions

An HbA1c level of ≥36 mmol/mol (≥5.4%), obtained close to the twenty-eighth week of pregnancy, was associated with a

more than 5-fold increased risk of diabetes five years after pregnancy. A cut-off level for HbA1c of ≥39 mmol/mol (≥5.7%), corresponding to the pre-diabetes range outside of pregnancy, could reveal women with post-partum diabetes with high specificity (97%) and high PPV (91%). Due to the low sensitivity, HbA1c does not appear suitable as a screening test to predict diabetes after GDM in all women, but it could be used as a strategy for selecting high-risk women for lifestyle interventions to prevent diabetes, starting already in pregnancy. The results should encourage further validation in large-scale studies using new diagnostic criteria for GDM.

Conflict of interest

The authors state that they have no conflict of interest.

Authors' contributions

KB conceived the study. All the authors contributed to the study design and interpretation of data. RC wrote the initial draft of the manuscript, and KB contributed to the next draft. CI and NS critically reviewed and edited the manuscript. All the authors approved the final version.

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Paper V

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Research Article

Seasonal Pattern in the Diagnosis of Gestational Diabetes Mellitus in Southern Sweden

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Aim. The aim of this study was to examine seasonal patterns in glucose tolerance and in the diagnosis of gestational diabetes mellitus (GDM). *Methods*. Altogether, 11 538 women underwent a 75-g oral glucose tolerance test (OGTT) in the twenty-eighth week of pregnancy during the years 2003–2005 in southern Sweden. GDM was defined by the 2-h capillary glucose concentration in the OGTT (≥8.9 mmol/L). Chi-squared test, analysis of variance, and regression analyses were used for statistical evaluations. *Results*. The seasonal frequency of GDM ranged from 3.3% in spring to 5.5% in summer ($p < 0.0001$). Mean 2-h glucose concentrations followed the same seasonal trend, with a difference of 0.15 mmol/L between winter and summer ($p < 0.0001$). The 2-h glucose level increased by 0.009 mmol/L for every degree increase in temperature ($p < 0.0001$). In regression analysis, summer (June–August) was associated with increased 2-h glucose level ($p < 0.001$) and increased frequency of GDM compared to the other seasons (odds ratio 1.51, 95% confidence interval 1.24–1.83, and $p < 0.001$). *Conclusions*. Our findings suggest seasonal variation in the 2-h glucose concentration in the OGTT and in the proportion of women diagnosed with GDM, with a peak in the summer.

1. Introduction

While seasonality in the onset of type 1 diabetes is well documented [1], less is known about seasonality in the diagnosis of type 2 and gestational diabetes mellitus (GDM). Doró et al. (2006) reported an increased incidence of type 2 diabetes onset in winter [2]. A similar pattern in HbA1c and glucose levels has been reported in diabetes patients, possibly because of seasonal variations in environmental conditions, such as diet and exercise [3–6]. In contrast, Schmidt et al. (1994) reported a 4-fold increase in the frequency of GDM in the summer compared with the winter, which they related to increased 2-h glucose levels in the oral glucose tolerance test (OGTT) at higher ambient temperatures [7]. Similar results of temperature-induced differences in post-load venous glucose levels have previously been reported by Akanji et al. in subjects with and without diabetes [8, 9]. However, two other studies from Australia and the UK found no clinically significant evidence of any seasonal variation in the prevalence of GDM or the 2-h glucose levels in the OGTT [10, 11].

The prevalence of GDM in a given population depends on the screening approach and on the diagnostic criteria used [12]. In southern Sweden, universal screening for GDM with a 75-g OGTT has been performed at the antenatal clinics since 1995, with no major changes in the diagnostic procedure. The diagnosis of GDM is based on the 2-h capillary glucose concentration [13]. During the years 2003–2005, pregnant women, representing different glucose categories according to the OGTT, were invited to take part in a follow-up study postpartum, the Mamma Study. During the period of recruitment, a large number of test results were made available and they form the basis of the present study. The aim was to determine whether there were any differences

in glucose tolerance by season and, consequently, if the frequency of GDM showed any seasonal variation. Such differences are important to elucidate, since they may affect the diagnostic procedure and interpretation of the results.

2. Materials and Methods

2.1. GDM Screening. The screening programme for GDM in southern Sweden has been described in detail elsewhere [13]. Briefly, a 75-g OGTT is offered to all women in the twenty-eighth week of gestation after overnight fasting and also in week 12 if there is a history of GDM in previous pregnancies or a first-degree relative with diabetes. The HemoCue blood glucose 201+ system (HemoCue AB, Ängelholm, Sweden) is used to perform immediate analysis of capillary glucose concentrations, which are reported as equivalent plasma glucose concentrations [14]. To ascertain the quality of the individual testing, double sampling is used, with acceptance of a divergence of ≤0.3 mmol/L. If this is not reached, a third sample is taken, and if the divergence between two of the samples is still not acceptable, the equipment is checked and the OGTT is not regarded as being valid.

The diagnostic criteria for GDM used in the present study are a slight modification of those recommended by the World Health Organization in 1999, defining GDM as a 2-h venous plasma glucose concentration of ≥7.8 mmol/L, corresponding to a capillary plasma glucose concentration of ≥8.9 mmol/L [15]. The diagnosis was based on the mean of the two measurements.

2.2. Study Population. Recruitment to the Mamma Study took place during the years 2003–2005 and involved four of the five delivery departments in the county of Skåne in southern Sweden [16]. During the recruitment period, OGTT results from the local antenatal clinics were sent to the study coordinator, enabling identification of the test results of women who consented to be enrolled; it also ensured correct sampling technique [13]. Initially, 11 976 OGTT results in total were reported. If a woman had more than one pregnancy during the study period, only the first one was included. Likewise, if a woman underwent more than one OGTT during the same pregnancy, only the one performed in pregnancy week 28 was included. This restricted data set (n = 11 538) formed the basis of the present evaluation. Mean monthly temperatures during the study period were obtained from the Swedish Meteorological and Hydrological Institute (http://opendata-download-metobs.smhi.se/explore/?parameter=3#).

The study was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants, and the study protocol was approved by the Ethics Committee of Lund University (LU 259-00).

2.3. Statistical Analysis. OGTT results from the 3-year study period were grouped together into months and seasons (winter: December–February; spring: March–May; summer: June–August; autumn: September–November). Chi-squared test was used to test for differences in frequencies between months and seasons, and one-way analysis of variance (ANOVA) was used to test for the corresponding differences in means. Multivariable logistic regression was used to examine whether month or season was associated with the diagnosis of GDM and multivariable linear regression was used to examine the corresponding associations with 2-h glucose levels. The relationship between mean monthly temperatures and mean monthly 2-h glucose concentrations was evaluated by simple linear regression.

IBM SPSS Statistics 22 for Windows (IBM Corporation, Armonk, NY) was used for analysis. Two-sided p values of less than 0.05 were considered to be statistically significant.

3. Results

Of the 11 538 women who underwent an OGTT during the study period, a total of 487 women (4.2%) were diagnosed with GDM.

Table 1 shows the study material, organized by month and season. The monthly frequency of GDM ranged from 2.9% in March to 5.8% in June, and the seasonal frequency of GDM ranged from 3.3% in spring to 5.5% in summer. The differences in frequencies were statistically significant, both for month ($p = 0.01$) and for season ($p < 0.0001$). The mean age of participating women was 29.9 (standard deviation 5.1) years, and the ages ranged from 15 to 49 years. The age of the women differed statistically significantly between months and seasons ($p < 0.001$). However, no significant differences in the monthly distributions of age were noted.

Mean monthly temperature ranged from −0.6°C in winter to 17.7°C in summer (Table 1). In a simple linear regression with 2-h plasma glucose as the dependent variable and mean monthly temperature as the predictor variable, the coefficient in the equation was 0.009, suggesting that the 2-h glucose level increased by 0.009 mmol/L for every degree increase in temperature ($p < 0.0001$).

Figure 1 illustrates the monthly mean 2-h glucose level during the OGTT (with 95% confidence interval (CI)) and the monthly percentage of women with GDM. Though numerically small, the differences in 2-h glucose levels were statistically significant ($p < 0.001$), with the lowest values observed from January to March and peak levels from June to August. A similar seasonal trend was seen for the percentage of women with 2-h glucose levels in the GDM range (2-h glucose level ≥ 8.9 mmol/L). There were no differences in the distribution of glucose concentrations between months or seasons.

In regression analysis, adjusting for age, the summer months (June to August) were found to be associated with increased 2-h glucose levels ($p < 0.001$) and increased frequency of GDM compared to all other months (OR = 1.51 (95% CI: 1.24–1.83); $p < 0.001$).

4. Discussion

In this observational study of 11 538 pregnancies, we found seasonal variations in the 2-h glucose level in the OGTT performed in the twenty-eighth week of gestation, giving

Table 1: Description of the study material, organized by month and season.

	OGTT *n*	GDM *n* (%)	2-h glucose, mmol/L Mean (SD)	Age, years, mean (SD)	Temperature, °C, mean
Month					
January	1 094	36 (3.3)	6.4 (1.3)	30.4 (5.0)	0
February	928	34 (3.7)	6.4 (1.3)	30.1 (5.3)	−0.6
March	1 082	31 (2.9)	6.4 (1.2)	30.1 (5.2)	2.4
April	1 027	34 (3.3)	6.5 (1.3)	30.1 (5.0)	7.5
May	1 057	41 (3.9)	6.5 (1.4)	30.4 (5.1)	12.1
June	1 009	59 (5.8)	6.6 (1.4)	29.8 (5.1)	15.2
July	974	50 (5.1)	6.6 (1.3)	30.0 (5.0)	17.7
August	928	52 (5.6)	6.6 (1.3)	29.6 (5.0)	17.6
September	781	33 (4.2)	6.5 (1.3)	29.5 (5.3)	14.4
October	835	42 (5.0)	6.6 (1.3)	29.6 (5.1)	8.4
November	897	38 (4.2)	6.6 (1.3)	29.6 (5.2)	5.3
December	926	37 (4.0)	6.5 (1.3)	29.8 (5.1)	2.9
Season					
Winter	2 948	107 (3.6)	6.4 (1.3)	30.1 (5.1)	0.7
Spring	3 166	106 (3.3)	6.5 (1.3)	30.2 (5.1)	7.3
Summer	2 911	161 (5.5)	6.6 (1.4)	29.8 (5.0)	16.8
Autumn	2 513	113 (4.5)	6.6 (1.3)	29.6 (5.2)	9.2

GDM, gestational diabetes mellitus; OGTT, oral glucose tolerance test; SD, standard deviation.

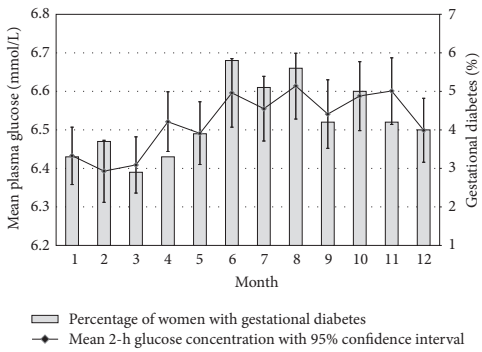

Figure 1: Monthly mean 2-h glucose levels and the monthly percentage of women with gestational diabetes mellitus.

seasonal variations in the percentage of women diagnosed with GDM—with a peak in the summer.

Until recently, there have only been three previous studies in the literature evaluating seasonality in GDM [7, 10, 11]. Using a 2-h venous plasma glucose concentration of ≥7.8 mmol/L to define GDM during a standardized 75-g OGTT in 1 113 consecutively tested women in Brazil, Schmidt et al. (1994) reported that the frequency of GDM varied in relation to the ambient temperature, from 4.8% in winter to 14.8% in summer [7]. For every degree increase in temperature, the mean 2-h glucose level increased by 0.07 mmol/L, while the fasting glucose levels were unaffected by temperature. Standardizing the results at 23°C indicated that women were overdiagnosed by 19% at higher temperatures and underdiagnosed by 45% at lower temperatures [7]. Similar results have been described in small-scale studies outside of pregnancy [8, 9]. Increased arterialisation of the venous blood at elevated temperatures has been suggested to be a plausible explanation [17]. Whether these variations in glucose levels result from an acute effect of temperature rather than a chronic one is not fully understood, although some experimental studies have indicated an acute effect [18, 19]. Interestingly, in a very recent study from the coastal area of Australia, Moses et al. reported significantly lower median 1-h and 2-h glucose levels in the OGTT in the winter compared with the overall 1-h and 2-h results, when evaluated in a cohort of 7 369 pregnant women prospectively followed up during a 3-year period [20]. Furthermore, in a population-based study from South Australia, Verburg et al. recently reported seasonal variation in the diagnosis of GDM based on the estimated date of conception [21].

Since the present study was based on capillary glucose measurements, previous findings cannot be directly extrapolated to results using our methodology. However, temperature-induced changes in peripheral blood flow may very well affect the composition of capillary blood as well, representing a mixture of arterial and venous blood. According to national statistics, the average temperature in the region varied between −0.6°C in winter and +17.7°C in summer during the study period. With respect to the simple linear regression analysis, indicating that for every one degree increase in temperature the glucose concentration

increased by 0.009 mmol/L, this difference in temperature between summer and winter corresponds to a difference of 0.15 mmol/L in glucose concentration. It is important to note that OGTT as such has a rather low reproducibility, especially for 2-h glucose levels in the intermediate range [22–24]. This means that a difference of 0.15 mmol/L would have diagnostic implications in women with glucose concentrations close to the diagnostic limit, thereby increasing the frequency of GDM in the summer.

Though not regarded as a diagnostic standard [25], capillary finger-tip tests have been used in the screening programme for GDM in southern Sweden ever since they were first introduced in 1995 [13]. For practical reasons, this was regarded as a prerequisite when introducing the OGTT on a large scale. Glucose measurements based on capillary samples are generally believed to show greater variation than venous glucose measurements [26]. If the hand is cold there is a risk of squeezing and "milking" of the finger, increasing the proportion of extracellular fluid, resulting in a lower glucose concentration. However, since glucose measurements in the present study were based on measurements after two hours at room temperature in the antenatal clinic, such an explanation would seem less likely.

In contrast to our findings and those of Schmidt et al. [7], Janghorbany et al. (2006) could not prove any seasonality in glucose tolerance or in the incidence of GDM in 4 942 pregnancies in Plymouth, UK [11]. However, it is important to note that Plymouth has a very mild climate, with little seasonal variation. Furthermore, only 11% ($n = 539$) of the women underwent an OGTT during pregnancy and the incidence figures were based on those with an abnormal OGTT ($n = 90$), as opposed to all the others who either had a normal OGTT or initially screened negative by random plasma glucose testing or risk factors. In a study from Australia, Moses and Griffiths (1995) examined seasonal trends in 2 749 women consecutively tested with a 75-g OGTT [10]. Interestingly, after adjustments for various risk factors, multiple regression analysis revealed a significant association between the monthly temperature and the 2-h glucose level in the OGTT; the 2-h glucose level increased by 0.026 mmol/L for every degree increase in temperature [10]. However, Chi-squared analysis did not suggest any seasonal variation in the diagnosis of GDM, and it was concluded that the association between 2-h glucose and temperature was unlikely to be of any clinical importance in the temperate coastal area of Australia, with the mean monthly temperature during the study period ranging from 13.6°C in July to 22.3°C in January [10].

Our results do not agree with previous observations by Doró et al. from Hungary, of an increased incidence of type 2 diabetes in winter, although it should be noted that the incidence figures reported were based on the initiation of antidiabetic therapy in individuals with previously diagnosed diabetes, therefore not representing the "true" diabetes onset [2]. Likewise, worsening of metabolic control in subjects with type 2 diabetes in winter has been described in a number of studies [3–6]. Since diet and exercise are hallmarks of the treatment of type 2 diabetes, it is reasonable to assume that environmental factors, such as diet and exercise patterns, have an important role in the seasonal variation in glucose metabolism in patients with diabetes. Seasonal variation in the diagnosis of GDM possibly reflects seasonality of environmental influences early in gestation, during placental development, affecting placental metabolism and glucose homeostasis later on in pregnancy [21]. Many factors vary with season, including the nutritional quality of foods, temperature, the number of hours of sunshine, and vitamin D synthesis. Maternal vitamin D deficiency in early pregnancy has been associated with increased risk of GDM [27]. Moreover, seasonal variation in vitamin D status, quantified as the total number of hours of sunshine during the three months preceding the onset of diabetes, was suggested as an explanation for the seasonality of type 2 diabetes reported by Doró et al. [28].

The strength of the present study was the uniform diagnostic procedure for GDM, based on universal screening with a 75-g OGTT and enabling identification of a large cohort of women in one particular week of gestation. Unfortunately, we did not have access to individual data other than age, so the figures are crude and unadjusted for other potentially important risk factors, such as body mass index and ethnicity [29]. However, we have no reason to believe that this would have any major effect on the results, due to the size of the material and the exclusive use of OGTT. Another weakness of the study was that we only had information on mean monthly temperatures during the study period and not the mean temperature on specific days, which makes it difficult to draw any firm conclusions on the effect of temperature on our results.

We have previously shown that approximately 5-6% of women with GDM in our region have positive islet cell autoantibodies as a marker of autoimmune pathogenesis and therefore of type 1 diabetes [30, 31]. Of these, 50% had developed type 1 diabetes within ten years postpartum [30]. Seasonality in the incidence of type 1 diabetes has been described [1], but this is probably not of any relevance to the present findings, due to the overall low proportion of women who would be expected to have autoimmune diabetes in our material.

5. Conclusions

Based on a universally performed OGTT in the twenty-eighth week of pregnancy, seasonality in the proportion of women diagnosed with GDM was observed, with a peak in the summer. The mean 2-h glucose concentration in the OGTT followed the same seasonal trend. The findings may be related to the increased ambient temperature in the summer. Further studies are needed to determine whether our results are reproducible and if they are, to investigate the cause(s) of seasonality, as such variations may have implications for the diagnostic procedure and for interpretation of the results.

Disclosure

An earlier version of this work was presented as an abstract at 2016 European Association for the Study of Diabetes (EASD) annual conference in Munich.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Kerstin Berntorp conceived the study. All the authors contributed to the study design and interpretation of data. Kerstin Berntorp wrote the initial draft of the manuscript, and Anastasia Katsarou and Rickard Claesson contributed equally to the subsequent draft. Claes Ignell helped analyse data. Claes Ignell and Nael Shaat critically reviewed and edited the manuscript. All the authors read and approved the final manuscript.

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Gestational diabetes mellitus

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The studies were conducted from 2012 through 2017:

I. The frequency of gestational diabetes mellitus with different criteria.
II. The performance of HbA1c, for diagnosis and/or screening, during the OGTT at GDM follow-up postpartum.
III. The relative importance of BMI and glucose levels in prediction of LGA births.
IV. Prediction of postpartum diabetes with HbA1c assessed during OGTT in pregnancy.
V. Seasonality of GDM.

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