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CHAPTER 1
INTRODUCTION

GESTATIONAL DIABETES MELLITUS

Gestational diabetes mellitus (GDM) is defined as any glucose intolerance with onset or first recognition during pregnancy. Recently, the definition has been updated to diabetes diagnosed in the second or third trimester of pregnancy that is not clearly overt diabetes. Type 1 or 2 diabetes mellitus (T1DM or T2DM) diagnosed before pregnancy, are referred to as pregestational diabetes and convey higher maternal and neonatal risk (1).

Epidemiology

GDM is the most common metabolic disorder during pregnancy with an estimated prevalence of 1–14%, depending on the population studied and the diagnostic criteria used. GDM accounts for the vast majority of pregnancies affected by diabetes mellitus (1). The prevalence is increasing worldwide in line with the obesity and T2DM epidemic, with major implications for public health (2). Risk factors for GDM include: advanced maternal age, body mass index (BMI) > 30 kg/m^2, previous pregnancy with GDM, previous child with birth weight > 4500 gram or > 95th percentile, history of polycystic ovary syndrome, history of unexplained intra-uterine death, and family history of diabetes mellitus and certain ethnic risk groups (e.g. Asian, Caribbean) (2,3).

(Patho)physiology

In pregnancy, insulin secretion increases in the first trimester whereas insulin sensitivity remains unchanged. From the second trimester onwards, insulin sensitivity progressively decreases to levels that approximate insulin resistance seen in type 2 diabetes mellitus (T2DM). Placental hormones such as progesterone, oestradiol, cortisol, prolactin and human placental lactogen, released mid-pregnancy contribute to the insulin resistant state (4). The development of insulin resistance serves as a physiological adaptation of the mother to ensure adequate nutrient supply for the rapidly growing foetus (5).

To compensate, a 2- to 2·5-fold increase in insulin secretion is necessary to maintain glucose levels within the normal range (5). GDM develops when pancreatic β-cells are unable to increase insulin secretion to levels that are sufficient enough to counteract the corresponding fall in insulin sensitivity.

Obesity, inflammation and GDM

Obesity is one of the greatest public health problems of the 21st century (6). Obese pregnant women are three times as likely to develop GDM as non-obese individuals (7). Pregravid obesity and excessive gestational weight gain (GWG) are often observed in women with GDM. Both are independent predictors of adverse pregnancy outcome (8). GDM and obesity combined have an even greater effect on pregnancy complications.

Adipose tissue does not only function as an energy storage entity but also as a biologically active endocrine organ, secreting adipokines (i.e. adiponectin, leptin) and inflammatory markers (i.e. tumor necrosis factor- alpha (TNF-α), interleukin-6 (IL-6)). Obesity is characterized by an altered production of inflammatory markers and adipokines causing a state of chronic low-grade inflammation (9).

Obesity and inflammation are increasingly being recognized as pathophysiological features of GDM (10). As a result, there is an increased interest in adipokines and other biomarkers in understanding the pathophysiology of GDM. The detection of biomarkers before the onset of hyperglycaemia may aid in the identification of women at risk.

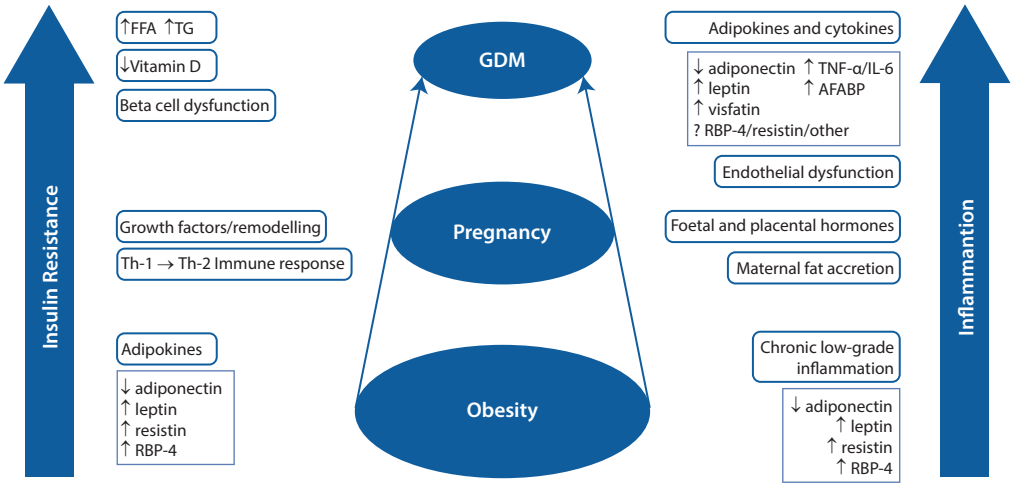

Figure 1 | Proposed model of relation between obesity, inflammation and GDM

Extracted with permission from Abell SK. Inflammatory and Other Biomarkers: Role in Pathophysiology and Prediction of Gestational Diabetes Mellitus Int J Mol Sci. 2015 Jun; 16(6): 13442–134

Ghrelin

Ghrelin is a gastro-intestinal peptide hormone and the endogenous ligand for the growth hormone secretagogue receptor (GHSR)1a. Total serum ghrelin levels are composed of acylated ghrelin (AG) and unacylated ghrelin (UAG). Ghrelin has a wide-range of biological activities and has been implicated in the regulation of glucose homeostasis (11). Furthermore, ghrelin or ghrelin mRNA is expressed in human ovary, testis and placenta, suggesting a role in fertility and pregnancy (12). It has been reported that ghrelin levels are lower in women with GDM, which may reflect the inhibitory effect of insulin on ghrelin secretion (13). Other studies found decreased ghrelin levels in pregnancy irrespective of glucose tolerance (14). However, to date, most studies measured total ghrelin, without differentiating between AG and UAG (15). The value of ghrelin as a biomarker in GDM needs to be evaluated with a double-antibody technique.

MATERNAL AND NEONATAL OUTCOMES

Pregnancies complicated by GDM are associated with an increased risk of adverse maternal and neonatal outcomes. These risks are related to uncontrolled hyperglycaemia. Data from the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) Trial established a linear relationship between maternal glucose levels and pregnancy complications (16).

Women with GDM are at increased risk of pregnancy-induced hypertension, preeclampsia and caesarean section. Moreover, women have a ~30% risk of GDM recurring in a subsequent pregnancy with higher rates observed in non-white populations (17,18). After pregnancy, hyperglycaemia resolves in most cases. However, women have an increased risk of T2DM and cardiovascular disease later in life (19). Evidence has shown that the risk of T2DM might be as high as 50% in the 5–10 years postpartum (20).

Maternal hyperglycaemia leads to increased transfer of glucose, lipids, and amino acids via the placenta. Maternal insulin cannot cross the placenta which results in foetal hyperinsulinaemia (21). This leads to fat accumulation, with insulin acting as a growth factor, stimulating intrauterine growth (22,23). Thus, foetal hyperinsulinaemia results in excessive growth of the foetus, leading to macrosomia (birth weight greater than 4000 g) or large-for-gestational-age (LGA) defined as birth weight > 90th percentile. This in turn is related to a higher frequency of birth trauma including shoulder dystocia, nerve palsies and clavicle fractures (16). Other neonatal complications include premature birth, neonatal hypoglycaemia, neonatal hyperbilirubinaemia (jaundice) and respiratory distress syndrome (24). Long-term risks in offspring include an increased risk of obesity and T2DM (25-27).

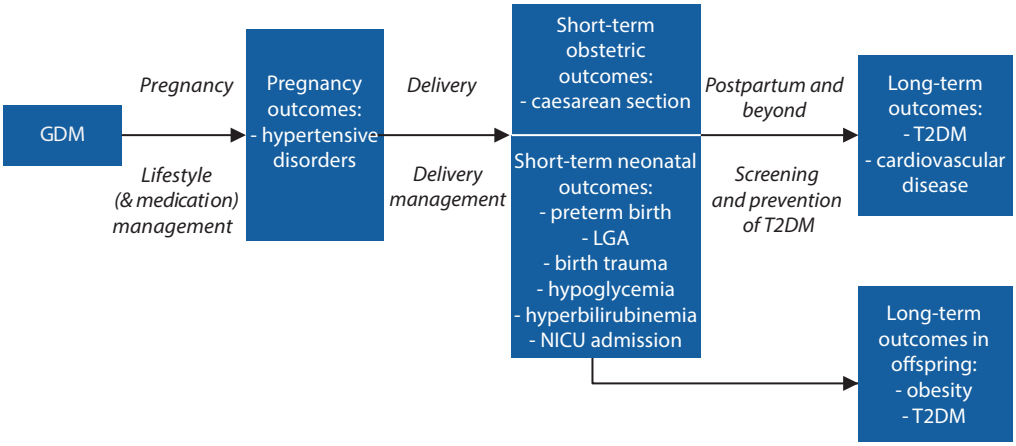

Figure 2 | Short and long-term outcomes of GDM

SCREENING METHODS AND DIAGNOSTIC CRITERIA

Screening for GDM is generally performed in women with risk factors or in case of symptoms (e.g. polyhydramnios or suspected foetal macrosomia) by means of an oral glucose tolerance test (OGTT). This test requires women to ingest a glucose solution containing 75-g of glucose after an overnight fast. Before and after administration of the glucose containing solution, plasma glucose values are measured. The approach to screening for GDM varies among leading international organisations and even within countries. Some expert groups recommend screening based on the presence of risk factors, while others state that this method fails to adequately detect all women with GDM and advocate universal screening (7,28,29?). Controversy regarding screening also exists due to lack of universally accepted diagnostic criteria and uncertainty about the threshold at which treatment becomes beneficial. O'Sullivan established the first diagnostic criteria in the 1960's and modified versions are still used to date. However, these criteria were designed to identify those at risk of T2DM after pregnancy and not those who are at risk of adverse pregnancy outcomes (30).

In 2010, the Internal Association of Diabetes and Pregnancy Study Groups (IADPSG) established new diagnostic criteria based on data from the HAPO trial (16,31). The IADPSG criteria are based upon an OR of 1.75 for negative pregnancy outcomes (75 g OGTT 0 h ≥ 5.1 mmol/l, 1 h ≥ 10.0 mmol/l, 2 h ≥ 8.5 mmol/l) and endorsed by global health organisations but not by the Dutch Society of Obstetrics and Gynaecology (3).

In the Netherlands, the national guideline "Diabetes and Pregnancy" recommends screening in women with one or more of the following risk factors: a first degree relative with diabetes mellitus; pre-gestational body mass index > 30 kg/m^2; previous child with birth weight > 4500 gram or > 95th percentile; history of unexplained intra-uterine foetal death, history of polycystic ovary syndrome and certain high-risk ethnicities (i.e. Afro-Caribbean, Hindu). Screening is performed by means of a 75-g OGTT between 24 and 28 weeks of gestation. Women with a history of GDM are screened between 16 and 18 weeks and 24 and 28 weeks of gestation. GDM is diagnosed if at least one value of plasma glucose level is equal to or exceeds the threshold (fasting glucose: ≥ 7.0 mmol/L and/or 2-hours post glucose load ≥ 7.8 mmol/L) (3). These diagnostic cut-off criteria are based on the World Health Organisation's (WHO) 1999 criteria (32).

MANAGEMENT OF GDM

Treatment

The treatment of GDM has been widely reviewed and proven to be effective in the reduction of adverse pregnancy outcome (33-35). Dietary and lifestyle intervention is the cornerstone of GDM treatment. For the majority of patients, optimal nutrition and a healthy lifestyle are sufficient to achieve glycaemic control. Treatment targets for GDM are: fasting glucose ≤ 5.3 mmol/L; 1-hour postprandial ≤ 7.8 mol/L; and/or 2-hours postprandial ≤ 6.7 mmol/L, capillary). If glycaemic targets are not met, then additional insulin therapy is the next form of treatment (3). The use of oral anti-diabetic agents in the treatment of GDM is gaining ground with data showing efficacy and safety

(36,37). However, long-term follow-up in children exposed to oral agents in pregnancy is required for widespread clinical acceptance (38,39).
Data on pregnancy outcomes under the current national screening and treatment guideline is scarce. Koning et al. showed that the number of adverse pregnancy outcomes in GDM was comparable with the general obstetric population in the northern region of the Netherlands (40). However, the incidence of LGA infants remained significantly increased. Most women in their GDM cohort were Caucasian women and is therefore not a reflection of all regions of the Netherlands.

Metformin

Metformin is a biguanide analogue, increasing insulin sensitivity and decreasing hepatic glucose production (41). Metformin is an emerging contender in the treatment of GDM (42). It is associated with less gestational weight gain and a lower risk of hypoglycaemia (43,44). Theoretically, metformin might reduce insulin resistance in pregnancy, causing a reduction in maternal glucose levels and subsequent foetal hyperinsulinaemia. In turn it could have a positive effect on the incidence of GDM and related pregnancy outcomes. To date, two large double-blind randomized-controlled trials (the EMPOWar trial and the MOP trial) showed no significant effect of metformin on birth weight percentile in obese pregnant women (45,46). However, other high-risk populations are yet to be investigated.

Monitoring

Treatment of GDM aims to maintain glucose levels equal to those of pregnant women without GDM. Therefore, insight into glucose regulation during the treatment of GDM is vital. Monitoring of treatment is currently based on self-monitoring of blood glucose (SMBG) by means of finger-stick glucose measurements. SMBG can reduce foetal overgrowth but the optimal frequency is unknown and compliance is low (47,48). Data has shown that 22% percent of women with GDM falsify or invent glucose values (49). Furthermore, SMBG does not provide a longitudinal glucose profile and could well hide periods of hyper-and hypoglycaemia. Blinded continuous glucose monitoring (CGM) is a monitoring technique which provides insight into the frequency and duration of hypo-and hyperglycaemic events (50). In patients with diabetes mellitus, CGM has shown the potential to aid clinical decision making in selected patient groups (51).

POSTPARTUM AND BEYOND

As previously mentioned, GDM is a risk factor for the development of T2DM and cardiovascular disease after pregnancy (52). With the increasing number of women with obesity and GDM, prevention of T2DM after pregnancy has become important. Lifestyle intervention and pharmacotherapy have both shown to reduce the incidence of T2DM in women with a history of GDM (53). Therefore, adequate diabetes screening programs are essential in this population. Postpartum screening attendance rates are low and little is known about long-term screening rates. In the Netherlands, diabetes screening is recommended annually in the first five years after pregnancy and every three

years thereafter (54). Data on adherence to these recommendations and the incidence of T2DM is limited.

SCOPE AND AIMS OF THIS THESIS

This thesis seeks to explore the overall management of GDM. The first aim is to investigate biomarkers and risk stratification. Which potential biomarkers can be used in the prediction of GDM? Is Ghrelin a useful biomarker? The second aim is to evaluate current management of GDM. Is the current national screening and treatment guideline for GDM effective in reducing GDM related complications? What is the role of blinded continuous glucose monitoring in treatment monitoring? The third aim is to study the adherence to long-term diabetes screening recommendations in women with GDM.

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PART I

BIOMARKERS AND PREDICTION

CHAPTER 2

The potential role of biomarkers in predicting Gestational Diabetes Mellitus

Brink HS, van der Lelij AJ, van der Linden J

Endocr Connect. 2016 Sep;5(5):R26-34

Introduction

Gestational diabetes mellitus (GDM) is defined as any glucose intolerance with onset or first recognition during pregnancy. GDM has a prevalence of ~7% worldwide, depending on the population studied and diagnostic criteria used (1). The incidence of GDM is increasing in line with the global rise of obesity and type 2 diabetes mellitus (T2DM) (2). GDM occurs when pancreatic β-cells cannot compensate for the increased levels of insulin resistance which occurs during pregnancy (3). Insulin resistance and β-cell dysfunction are two known mechanisms, however the exact cellular mechanisms remain to be elucidated (4). GDM is associated with maternal and neonatal short- and long-term complications (5,6). For the offspring this includes a predisposition for development of obesity and T2DM (7,8). Long-term maternal risks include T2DM and cardiovascular disease (9). Currently, GDM is diagnosed in the late second trimester, possibly exposing the infant to intra-uterine metabolic alterations and epigenetic programming for a significant period of time. Reported evidence suggests that metabolic alterations can predispose infants to long-term pathology (10,11). Detection and management of GDM in pregnancy can reduce the frequency of adverse pregnancy outcomes (12,13). Hence, there is need for improved prediction as current risk stratification fails to correctly identify all women with GDM (14,15).

Investigating the role of adipokines associated with the pathophysiology of GDM has gained interest (16,17). Adipokines have in recent years been posed as the link between adiposity and adverse complications such as insulin resistance. Identification of early biomarkers in pregnant women, who subsequently develop GDM, may result in improved understanding of GDM pathogenesis. Combining biomarkers and risk factors into a predictive model may add to early prediction of GDM, evoke effective prevention strategies and may ultimately reduce complications associated with GDM.

The aim of this review is to 1) identify potential predictive biomarkers in GDM and 2) discuss the role of incorporating predictive biomarkers into clinical risk prediction models, for the stratification of high-risk patients.

Epigenetic footprint

Metabolic alterations such as impaired glycaemic control during foetal development can lead to functional and structural alterations in the foetus, resulting in a predisposition for developing chronic metabolic diseases later in life. These alterations are also referred to as 'foetal programming' and they can cause epigenetic changes (10).

Epigenetic changes ascribe to the change in the biochemical structure of DNA that ultimately alters gene expression. This includes DNA methylation, histone modification and non-coding RNA processes (18). Epigenetic changes have been observed in many disease states and offer biochemical evidence of the detrimental effects of adverse developmental conditions and subsequent disease (10). This relationship has been supported by epidemiologic and animal studies (19-22). Furthermore, it has been reported that maternal insulin resistance also causes insulin resistance in the foetus, as early as the embryonic stage (23). Multiple studies have linked maternal GDM with the development of obesity and T2DM in children (11,24), who are eight-times more likely to develop T2DM than non-

GDM children (25). Therefore, there is a strong need for early detection of GDM. Detection preceding the hyperglycaemia might avoid subsequent harm. Investigating early predictive biomarkers in GDM may be a step in this direction.

Obesity, inflammation and GDM

More women of childbearing age are entering pregnancy being overweight or obese (26). Obese pregnant women have a three-fold risk for developing GDM (27). The global increase in GDM is largely attributed to the ongoing obesity pandemic (28). Obesity is characterized by altered production of proinflammatory cytokines by adipocytes causing a state of chronic low-grade inflammation (29), driving the expression and production of proinflammatory (tumor necrosis factor- alpha (TNF-α), interleukin-6 (IL-6)) and anti-inflammatory cytokines or adipokines (adiponectin, leptin, visfatin) (30). Adipokines have a clear regulatory role in metabolism, including modifying insulin secretion and sensitivity, appetite, energy control and inflammation (31). Clinical and epidemiologic studies have described a sound relation between obesity, chronic low-grade inflammation and the development of T2DM (32). In normal pregnancy, the immune system is subjected to changes with a delicate balance between production of pro- and anti-inflammatory cytokines. Pregnancies in obese individuals further enhance the proinflammatory profile leading to an imbalance and, therefore, possible complications. It is increasingly being recognized that inflammation is a pathophysiologic feature of GDM (33,34). In GDM, a pro-inflammatory state prevails and the increased production of proinflammatory cytokines debilitates insulin signalling (35). Down regulation of adiponectin and anti-inflammatory markers such as IL-4, IL-10 and upregulation of pro-inflammatory cytokines such as IL-6 and TNF- α can be observed in GDM (35,36).

Adipokines

Adiponectin

Adiponectin is an adipocyte-derived protein. It contains anti-atherogenic, anti-inflammatory and insulin-sentizing properties (37). Adiponectin is inversely correlated with obesity, hypertension, serum lipids and coronary artery disease (37,38). Decreased adiponectin levels have also been associated with an increased risk of T2DM (39,40). Adiponectin levels are known to decrease progressively during normal pregnancy, probably in response to decreased insulin sensitivity (41). Several studies have also shown reduced adiponectin levels during mid-pregnancy (24–28 weeks) in GDM compared to controls (42-47), relating low levels of adiponectin to the onset of insulin resistance and diminished β-cell function (48). A systematic review and meta-analysis of adiponectin concentrations in 560 GDM and 781 controls underlined a significantly decreased adiponectin level in women with GDM versus controls (47). However, it must be noted that results are in light of a significant heterogeneity among the included studies. In recent years, prospective studies have addressed the role of adiponectin as a possible early predictor of GDM. Lower levels of adiponectin in the first trimester of pregnancy are associated with a greater risk for developing GDM (49-51), suggesting that a down-regulation of adiponectin may be a predictor of GDM. However, in a systematic review and meta-analysis, adiponectin had a moderate effect for predicting future GDM with pooled diagnostic odds ratio (DOR) of 6.4 (95% CI 4.1, 9.9), a summary sensitivity of 64.7%

(95% CI 51.0%, 76.4%) and a specificity of 77.8% (95% CI 66.4%, 86.1%) (52). Furthermore, a nested-case control study showed that low pre-pregnancy adiponectin levels are associated with a 5.0-fold increased risk of developing GDM (53). This association remained significant after adjusting for known risk factors for GDM. This might be relevant for clinical practice as it identifies a group of high-risk women that might otherwise not have been identified. Adiponectin therapy has been tested in animal models of obesity and it has been shown to improve glycaemia and reduce hyperinsulinemia without alterations in body weight (54).
In summary, lower levels of adiponectin are linked to obesity, type 2 diabetes and GDM. Adiponectin might play a role in the pathophysiology of GDM and can be seen as a promising predictive biomarker for GDM. Further research addressing lifestyle interventions or adiponectin intervention therapy is needed to further establish the role adiponectin in GDM.

Leptin

Leptin is an adipocyte-derived hormone. It is predominantly produced by adipocytes but is also produced in ovaries and the placenta. It regulates energy balance through hypothalamic pathways (55). Increased leptin concentrations are associated with weight gain, obesity and hyperinsulinaemia (56). Maternal leptin levels are known to increase 2–3 fold in pregnancy, likely due to placental secretion (57). Increased leptin levels have been reported in women with GD (47). Inflammatory markers, such as IL-6 and TNF-α probably also play a role in the pathophysiology of GDM by promoting chronic low-grade inflammation, while further increasing leptin concentrations (58). A prospective cohort study reported increased concentrations of leptin before 16 weeks gestation, independent of adiposity, which were associated with an increased risk of GDM (59). Another small study showed that leptin was increased in all women during pregnancy, with highest concentrations observed in obese GDM subjects. Adjusted for fat mass, this correlation disappeared, however (35). Generally speaking, current evidence is limited, in part due to confounding effects of measures of adiposity. Leptin is likely to be involved in the pathophysiology of GDM but appears to be a poor predictor of GDM.

Visfatin

Visfatin is an adipokine and is mostly produced by visceral fat. It has endocrine, paracrine and autocrine actions (60). Increased visfatin levels have been reported in obesity, metabolic syndrome and T2DM (61,62). In pregnancy, visfatin levels progressively increase up to the second trimester, after which they decease again with the lowest concentrations observed in the third trimester (63). In GDM, reports on visfatin levels have thus far been inconsistent, as both decreased and increased levels have been reported (64-66). Another study showed that visfatin measured in the first trimester was better in the prediction of GDM compared to CRP, Il-6, adiponectin and leptin (67). In a case-control study, visfatin levels measured in the first trimester were increased in the GDM group but when added to other maternal risk factors, the detection rate for GDM did not improve (68). Results thus far suggest that visfatin is a potential biomarker in GDM, but additional prospective studies are definitely needed to further investigate the relationship between visfatin and GDM.

Resistin

Resistin is an adipose-derived hormone expressed by monocytes, macrophages and adipocytes (69). Resistin is positively associated with adiposity. Resistin levels are known to increase during pregnancy, probably due to weight gain (58,70). A potential link between resistin, adiposity and insulin resistance in pregnancy might exist but to date remains inconclusive due to conflicting reports from case-control studies (71,72). Nested-case control studies, investigating resistin levels in early pregnancy, found no differences in resistin levels between GDM and controls (adjusted for BMI) (36,51). A prospective study with larger sample size than the previous case-control studies also showed no significant association between resistin and GDM (73). Other studies have shown elevated maternal levels of resistin in GDM (70,71,74). A systematic review showed no significant association between resistin levels and GDM pregnancies (75). Significant heterogeneity among studies was a major issue in the analysis. Currently there is no sound evidence that resistin is involved in the pathophysiology or prediction of GDM.

Other inflammatory mediators

Tumor necrosis factor-alpha (TNF-α)

TNF-α is a pro-inflammatory cytokine and is produced by monocytes and macrophages. It affects insulin sensitivity and secretion through impairing β-cell function and insulin signalling pathways, resulting in insulin resistance and possibly GDM (76). Multiple studies have reported increased maternal TNF-α levels in subjects with GDM, predominantly in late pregnancy (77-79). A meta-analysis also showed increased TNF-α levels in GDM versus controls. Subgroup analysis revealed that this relation remained significant when compared to BMI-matched controls (47). The increased levels are thought to be due to increased oxidative stress and inflammation associated with the impaired glucose metabolism (80). A small nested-case control study with only 14 cases and 14 controls addressing the predictive value of TNF-α showed no differences between women with GDM and controls (36). In a prospective study in GDM and controls, TNF-α levels were measured pre-gravid, at 12–14 weeks and 34–36 weeks. TNF-α levels were increased at 34–36 weeks of gestation and were inversely correlated with insulin sensitivity (35). Further prospective studies are required to investigate the predictive value of TNF-α in GDM, adjusting for measures of adiposity.

High sensitivity C- reactive protein (hsCRP)

(hs)CRP is an acute-phase protein and produced in response to tissue injury, inflammation and infection. (hs)CRP has been shown to be associated with i.e. obesity and diabetes mellitus. In turn, it is well known that obesity is associated with inflammation, which contributes to insulin resistance. Elevated first trimester (hs)CRP levels are associated with GDM risk (*P* for trend=0.007). After adjusting for pre-pregnancy BMI, family history of DM and nulliparity, women with (hs)CRP in the highest quartile had a 3.5-fold increased risk of GDM as compared to those in the lowest quartile (34). Wolf et al. also reported that first-trimester CRP levels were significantly increased among women who subsequently developed GDM compared with control subjects (3.1 vs. 2.1 mg/L, $P < 0.01$). After adjusting for age, race/ethnicity, smoking, parity, blood pressure, and gestational age at CRP sampling, the increased risk of developing GDM among women in the highest compared with the

lowest tertile was 3.6 times higher (95% CI 1.2–11.4). When adjusted for BMI this association was not found anymore, however (81). Berggren et al. evaluated whether first-trimester (hs)CRP was predictive for third-trimester impaired glucose tolerance (IGT). (hs)CRP was positively associated with IGT but, again, the association disappeared when adjusted for BMI (82). Thus far, the positive association of (hs)CRP and GDM seems to be in part mediated by BMI.

Sex-hormone binding globuline (SHBG)

SHBG is a glycoprotein and plays a role in the regulation and transport of sex hormones. In vitro, SHBG has been proposed as a marker in insulin resistance as it has shown that insulin and insulin-like growth factor cause inhibition of SHBG secretion (83). Indeed, a relation between low levels of SHBG and T2DM has been reported (84). A prospective cross-sectional study evaluating the SHBG serum levels reported that SHBG concentrations were significantly lower in GDM subjects than in normal pregnancies (85). Furthermore, in women who were treated with insulin, SHBG levels were reported to be even lower (86). This might suggest that SHBG could help to differentiate or predict the women who will require insulin therapy. The overall additional clinical and predictive value of these results is limited as testing on GDM is already routinely performed at this stage of pregnancy. A prospective observational study (n=269) evaluating several biomarkers earlier than 15 weeks of gestation showed that low levels of SHBG were associated with an increased risk of GDM. This association was independent of other risk factors (BMI, smoking, blood pressure). Using the cut-off value of 211.5 mmol/L, SHBG showed an acceptable sensitivity of 85% but a low specificity of 37%. Adding (hs)CRP increases the specificity to 75.46%, however (87). Another prospective cross-sectional study, addressing the predictive value of SHBG for the diagnosis of GDM, reported that low levels of SHBG assessed between 13–16 weeks of gestation were positively associated with the development of GDM (n=30) ($P < 0.01$) (88). A limitation in this study, however, was that they could not establish an SHBG cut-off value for a constant term of pregnancy. A nested-case control study showed that non-fasting SHBG in the first trimester was consistently associated with an increased risk for GDM (17).

Other potential biomarkers

Adipocyte fatty acid-binding protein (AFABP) is an independent risk predictor for metabolic syndrome, T2DM and cardiovascular disease (89). Two studies have reported increased concentrations in GDM (90,91). Studies investigating the predictive value of AFABP in GDM have not been performed to date, however. IL-6 is a proinflammatory cytokine and is increased in obesity and associated with indices of adiposity and insulin resistance, such as body mass index (BMI) (92,93). Controversy exists regarding the changes in circulating levels of IL-6 in obesity. The relationship between IL-6 and insulin action appears to be mediated via adiposity (94). However, in a case-control study, plasma IL-6 levels have shown to be elevated when adjusted for BMI in women with GDM (95). Low levels of vitamin D have been associated in obesity and T2DM. In pregnancy, low levels are also often observed (96). Low vitamin D levels in the first trimester were also associated with a higher risk for GDM (adjusted for confounders and risk factors) (96). Recent meta-analyses have supported this finding, but the included studies were not all randomized controlled (97). Future RCTs are needed to further clarify the predictive role of vitamin D.

Clinical prediction models incorporating biomarkers

Current screening methods only identify women who already have impaired glucose metabolism. Ideally, subjects with high risk of GDM should be identified before they exceed the oral glucose tolerance test (OGTT) threshold values. Early prediction would allow for timely intervention that could limit gestational weight gain and obesity and possibly the onset of GDM. Current screening methods have moderate detection rates (98,99). Clinical risk prediction models have been investigated in GDM. For example, the development of GDM can be predicted from the ethnicity, family history, history of GDM and body mass index. The model showed an area under the receiver operating characteristic curve of 0.77 (95% CI 0.69–0.85) (100). If an OGTT was performed in all women with a predicted probability of 2% or more, 43% of all women would be tested and 75% of the women with GDM would be identified (100). Furthermore, in a large prospective cohort (n=7929), the best performing model, based on ethnicity, BMI, family history of diabetes and past history of GDM showed a sensitivity, specificity and AUC of 73% (66–79), 81% (80–82) and 0.824 (0.793–0.855), respectively, for the identification of GDM cases requiring insulin therapy (101). Introducing biomarkers to a set of clinical risk factors may enhance predication rates. For example, tissue plasminogen activator (t-PA) and low HDL cholesterol were independent significant predictors of GDM. The addition of these biomarkers to a set of demographic and clinical risk factors increased the area under the curve (ROC) from (0.824 to 0.861) (102). t-PA in the prediction of GDM is a novel finding but previous work has shown that t-PA is associated with an increased risk of T2DM (103). Another study demonstrated that elevated plasma insulin and reduced adiponectin levels in the first trimester improved GDM identification rates compared to clinical factors alone (36). Maternal risk factors alone showed a prediction rate of 61% for GD, adding adiponectin and SHBG increased detection rates to 74% (16). Investigators in another study showed that adding adiponectin to a set of clinical risk factors increased the area under the receiver-operating curve increased significantly (104). Adding maternal visfatin and adiponectin to a set of maternal risk factors showed a detection rate of 68% (95% CI 58.3–76.3%) (68). The clinical implementation of such multi-parametric prediction models depends on significant reduction in adverse pregnancy outcomes, practical acceptability and cost-effectiveness. Ultimately, these models require prospective validation studies and further identification of predictive threshold values for these biomarkers.

Conclusion

GDM is currently detected in late pregnancy, unnecessarily exposing the infant to harmful intrauterine conditions. There is a definite clinical need to better predict and detect GDM early in pregnancy in order to prevent further harm to mother and child. Adiponectin is probably one of the most promising candidates in the prediction of GDM. The clinical value of implementing a combined clinical model is questionable as the current level of evidence is weak due to study design, differences in diagnostic criteria and assay methods used. Well-designed prospective studies

addressing current limitations are needed to identify reliable predictive biomarkers in GDM and their additional value to current clinical prediction tools.

Declaration of interest statement

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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CHAPTER 3

The Ghrelin System and Gestational Diabetes Mellitus

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INTRODUCTION

Gestational diabetes mellitus (GDM) is associated with an adverse pregnancy and perinatal outcome. A variety of serum biomarkers (e.g. inflammatory cytokines, adipokines and other circulating proteins) have been explored in attempts to identify a reliable predictor in early pregnancy for subsequent development of GDM, but so far none have been found (1). Hence, the pool of biochemical markers should be explored further.

Ghrelin is a gastro-intestinal peptide hormone and the endogenous ligand for the growth hormone secretagogue receptor (GHSR)1a. Total serum ghrelin levels are composed of acylated ghrelin (AG) and unacylated ghrelin (UAG). The enzyme ghrelin O-acyltransferase (GOAT) is required for the acylation of ghrelin. Ghrelin seems to have a wide-range of biological activities and has been implicated in the regulation of glucose homeostasis (2).

Ghrelin or ghrelin mRNA is expressed in human ovary, testis and placenta, suggesting a role in fertility and pregnancy (3). The pathophysiological role of ghrelin in GDM remains unclear, however. It has been reported that ghrelin levels are lower in women with GDM, which may reflect the inhibitory effect of insulin on ghrelin secretion (4). Other studies found decreased ghrelin levels in pregnancy irrespective of glucose tolerance (5). However, to date, most studies measured total ghrelin, without differentiating between AG and UAG. Furthermore, single-antibody ghrelin assays recognize the COOH-terminal (total ghrelin) or the acylated NH_2-terminal part of the peptide (AG) and therefore measure full-length ghrelin as well as circulating fragments of ghrelin with unknown biological activity. It has been estimated that 60% of the ghrelin measured using these assays is fragmented (6). We used a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) assay that measures full-length AG and UAG. We hypothesized that women with GDM have a higher degree of insulin resistance and hyperinsulinemia, with subsequently lower ghrelin levels than women with normal glucose tolerance (NGT). The aim of this study was to compare AG, UAG levels and AG/UAG ratios between pregnant women with GDM and NGT.

METHODS & MATERIALS

Subjects

In the study 19 pregnant women with GDM and 19 women with NGT were enrolled. Women were prospectively recruited from the gynaecology outpatient clinic of the Maasstad Hospital, Rotterdam, the Netherlands. Women with a singleton pregnancy, ≥ 18 years old, gestational age between 24–28 weeks, and a high risk of GDM according to the Dutch Society of Obstetrics and Gynaecology (7), were eligible for inclusion. Women were excluded if they met the following criteria: endocrine disorders such as acromegaly, pre-existing type 2 diabetes mellitus, Cushing's syndrome or the use of glucocorticoid medication, inflammatory diseases or active infections, and history of gastrointestinal surgery or hormonal treatment before or during pregnancy, including insulin. All subjects were screened for GDM between 24–28 weeks of gestation by means of a 75-g oral glucose tolerance test (OGTT) and the diagnosis of GDM was based on the IADPSG diagnostic criteria (8).

Blood samples were drawn during fasting and 2-hours post glucose load. Patient demographics such as age, BMI (kg/m^2), first trimester glucose (mmol/L) and gestational age at sampling (weeks) were obtained from electronic medical records. All patients gave written informed consent before inclusion in this study. This study was approved by the medical ethics committee.

Materials

Vacutainers from Becton Dickinson (Breda, Netherlands; cat# 367899; 6 mL K2 EDTA) were used. 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (Pefabloc, SC AEBSF) was purchased from Roche Applied. Science (cat# 11429876001; Almere, Netherlands). Aliquots of 200 mg/mL stock solutions of AEBSF were prepared in distilled water and stored at -80°C for a maximum of 3 months. Human AG and UAG are determined by a double-antibody sandwich technique. The enzyme immunoassay (EIA) kits were obtained from Bertin Pharma (Montigny-le-Bretonneux, France; A05106 and A05119, respectively).

OGTT, sample collection and storage

After an overnight fast, the 75-g OGTT was performed. Baseline serum parameters were glucose (4ml heparin tube), HbA1c (8.5ml serum separating tube), AG and UAG (4ml EDTA). Two hours after glucose ingestion we assessed: glucose (4ml heparin tube) and AG and UAG (4mL EDTA). Immediately after sample collection, AEBSF (dilution 1:100) was added to the AG and UAG blood samples to prevent des-acylation of AG. Tubes were carefully mixed by inversion and stored on water ice (0°C) until centrifugation at 2500 g at 4°C for 5 min. Plasma samples were stored in 300 µL aliquots at -80°C until assayed for AG and UAG. After slowly thawing on water ice, all plasma samples were briefly cleared by centrifugation before transferring into the assay plates. All samples were analysed in duplicate (50µL/well) (9). A cubic polynomial fitting was used to determine concentrations from the calibration curves, resulting in $r^2 > 0.995$ for all assays performed. Intra-assay coefficient of variation (%CV) is typically 2.7% CV for AG and 3.4% CV for UAG. Inter-assay % CV is 13.2% CV and 15.0% CV, for AG and UAG respectively (manufacturer's suggested cut-off=25%).

STATISTICAL ANALYSIS

Comparative analysis between groups was calculated by Mann–Whitney U tests and Wilcoxon's signed rank test. Statistical analysis was performed using SPSS software (version 16 for Windows; SPSS, IBM, Armonk; NY; USA) and GraphPad Prism Version 6.04 (GraphPad Software; La Jolla; CA; USA). The results are expressed as median ± interquartile range (IQR). *P*-values of < 0.05 were considered statistically significant.

RESULTS

Patient characteristics are shown in Table 1. Obviously, women with GDM had a significantly higher HbA1c, fasting and 2-hour post glucose load levels than pregnant women with NGT (*P*=0.001).
Fasting median plasma AG levels in GDM (N=19) and NGT (N=19) were 11.5 pg/mL (IQR: 5.4–16.8) and 13.7 pg/mL (IQR: 10–25.6, *P*=0.456), respectively ('reference levels' of AG range from 22.7 to 61.9 pg/mL) (9). Median 2-hour post OGTT plasma AG levels in GDM and NGT were 10.4 pg/mL (IQR: 2.7–13.9) and 10.2 pg/mL (IQR: 6.0–13.6, *P*=0.672), respectively. Fasting UAG median plasma levels in GDM and NGT were 44.6 pg/mL (IQR: 37.8–84.2) and 72.8 pg/mL (IQR: 39–146, *P*=0.320), respectively ('reference levels' of UAG range from 23.3–33.4 pg/mL) (9). Median 2-hour post OGTT UAG levels in GDM and NGT were 34.4 pg/mL (IQR: 26–49.3) and 46 pg/mL (IQR: 26.4–99.6, *P*=0.293) respectively. The fasting median AG/UAG ratios in GDM were 0.2 (IQR: 0.13–0.31), which were not significantly different from the median AG/UAG ratios in NGT 0.19 (IQR: 0.14–0.28), *P*=0.605). The 2-hour post glucose load AG/UAG ratios in GDM and NGT were also similar 0.23 (IQR: 0.11–0.29) and 0.19 (IQR: 0.14–0.28) *P*=0.736), respectively.
Plasma AG concentrations decreased significantly 2-hour post OGTT in GDM (*P*=0.001) as well as in NGT (*P*=0.002) (Figure 1A). Plasma UAG also decreased significantly 2-hour post OGTT both in GDM (*P*=0.0001 and in NGT (*P*=0.0002) (Figure 1B). There were no significant differences in plasma AG/UAG ratios post glucose load between GDM (*P*=0.776) and NGT (*P*=0.827).

Table 1 | Patient characteristics

Parameter	GDM N=19	NGT N=19	*P*-value
Age – yr	35 (30–38)	34 (29–37)	0.320
BMI – kg/m^2	28.4 (25–35)	29.7 (25–35)	0.942
Pre-pregnancy BMI – kg/m^2	28 (24–35)	29 (23.4–35)	0.827
First trimester glucose – mmol/L	5.1 (4.4–5.3)	4.7 (4.3–5.4)	0.428
Gestational age at blood collection – weeks	24 (24)	24 (24–25)	0.139
75-g OGTT: fasting glucose – mmol/L	5.3 (5.1–5.8)	4.6 (4.3–4.8)	0.001*
75-g OGTT: 2-hour glucose – mmol/L	8.8 (7.1–9.9)	6.2 (5.5–7.0)	0.001*
HbA1c – mmol/mol	35 (33–38)	31 (29–32)	0.001*

BMI: Body Mass Index; OGTT: Oral Glucose Tolerance Test
Data are presented as median with inter-quartile range. *$P < 0.05$ was considered statistically significant.

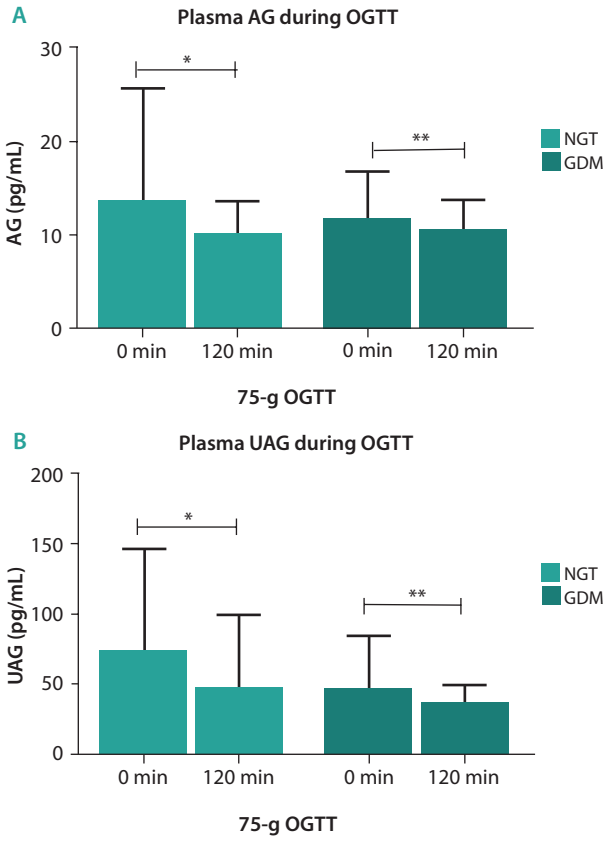

Figure 1 | Ghrelin levels and response to oral glucose tolerance test

(A) Plasma acylated ghrelin (AG) levels during OGTT in women with normal glucose tolerance (NGT) and gestational diabetes mellitus (GDM). Data are shown as median with interquartile range. * *P*=0.002, ***P*=0.001.

(B) Plasma unacylated ghrelin (UAG) levels during OGTT in women with normal glucose tolerance (NGT) and gestational diabetes mellitus (GDM). Data are shown as median with interquartile range. * *P*=0.0002, ** *P*=0.0001.

Discussion

In contrast to our hypothesis, and using a sensitive assay, plasma ghrelin levels were not lower in women with GDM compared to women with NGT. Ghrelin levels decreased significantly 2-hour post OGTT in both groups, which suggests that the physiological negative effect of oral glucose intake on ghrelin levels is still intact. These results indicate that ghrelin is not a useful biomarker in GDM. Our data are in agreement with the results of a study reported by Riedl et al. who found no association between fasting or post-load plasma ghrelin levels and reported that ghrelin suppression in GDM is not the result of insulin resistance. These authors suggest that ghrelin suppression is required

to allow the physiological insulin resistance necessary for growth and nourishment of the foetus (5). Another study by Telejko et al. found decreased ghrelin levels during pregnancy irrespective of glucose tolerance status (3). On the contrary, studies have indeed reported lower ghrelin levels in GDM compared to healthy pregnant women. For example, Palik et al. showed that serum AG levels, using a different assay than we did, were significantly lower in women with GDM versus NGT during the third trimester of pregnancy (10).

Ghrelin levels appeared suppressed during pregnancy in comparison with reference levels from healthy non-pregnant individuals (9). Likewise, Tham et al. showed that ghrelin levels were suppressed relative to postpartum, using a similar assay (4). Furthermore, these authors showed that AG levels recovered after pregnancy, which implies that the low AG/UAG ratio is a result of pregnancy.

Pregnancy is characterized by increased food intake, maternal weight gain and progressive insulin resistance. The orexigenic effects of ghrelin may contribute to the positive energy balance, while adipose tissue causes a negative feed-back regulation on ghrelin production (10). Ghrelin levels decrease with hyperglycaemia and hyperinsulinaemia, reflecting the inhibitory effect of insulin on ghrelin secretion (4). This hypothesis is supported by increased ghrelin levels during mid-pregnancy and decreased levels during late gestation (11). Whether low ghrelin levels are a risk factor or a compensatory mechanism, is unknown.

The strength of our study was that we were able to measure both AG and UAG with a sensitive assay, but it has also some limitations. Firstly, comparing our results with other study outcomes is difficult because of the different assay techniques used. Secondly, a larger sample size might improve the reliability of the outcome, although our data show no suggestion of an association of ghrelin levels in early pregnancy with the development of GDM in the population of subjects studied.

In conclusion, both AG and UAG levels are low during pregnancy regardless of the level of glycaemic control, and they decrease normally after an oral glucose load.

DECLARATION OF INTEREST

Aart Jan van der Lely is a co-founder and shareholder of Alizé Pharma SA; Ecully, France.

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PART II

EVALUATING CURRENT MANAGEMENT

CHAPTER 4

Maternal and neonatal outcomes of Gestational Diabetes Mellitus

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Submitted for publication

Abstract

Background: To analyse maternal and neonatal outcomes of gestational diabetes mellitus (GDM) and identify factors associated with neonatal complications.

Methods: An analysis of singleton GDM pregnancies treated for GDM in a multi-ethnic cohort in Rotterdam, the Netherlands between 2010 and 2015. Outcomes were compared to a control group (574.823) with no GDM or pregestational diabetes from the Netherlands Perinatal Registry.

Results: We analysed 1008 consecutive singleton pregnancies with GDM. Insulin therapy was required in addition to diet and lifestyle counselling in 389 women (38.7%). The rates of pre-eclampsia (3.8% vs. 5.1%), large-for-gestational-age (> 90th percentile) (8.5% vs. 10.7%) and pre-term birth (6.6% vs. 8.1%) were not significantly different between groups. The rate of labour induction was higher than in the CG (56% vs. 29.2%). The composite neonatal outcome included the following neonatal complications: death (perinatal/neonatal), neonatal hypoglycaemia (< 2.6 mmol/L), admission to neonatology department, hyperbilirubinaemia requiring phototherapy or birth trauma (shoulder dystocia, brachial plexus injury, bone fracture (humerus/clavicle) and occurred in 272 (27%) neonates. Independent risk factors predicting the composite neonatal outcomes were body mass index (kg/m^2) ≥ 30 (OR 1.6 [1.1–2.3] *P*=0.041) and insulin therapy (OR 2.5 [1.7–3.9] *P*=0.001).

Conclusions: In a large multi-ethnic cohort in Rotterdam, treatment of GDM according to the current guideline results in a low rate of adverse maternal and neonatal outcomes. Obesity and insulin therapy are independently predictive of an increased risk of neonatal complications. This study underlines the importance of weight control before pregnancy. Therefore, health care professionals should play an important role in prevention of obesity in young women.

Introduction

Gestational diabetes mellitus (GDM) is defined as any carbohydrate intolerance with onset or first recognition during pregnancy. GDM occurs in 1–14% of all pregnancies, depending on the population studied and diagnostic criteria used (1). Worldwide, the prevalence of GDM is increasing, and this global increase is mostly attributed to the obesity epidemic (2). GDM is associated with an increased risk of adverse pregnancy and perinatal outcome such as preeclampsia, caesarean section and macrosomia (3). Women with a history of GDM are also at increased risk of developing Type 2 diabetes mellitus later in life (4). Long-term adverse health outcomes for offspring include increased risk of obesity and Type 2 diabetes mellitus (5,6). Current treatment of GDM consists of lifestyle and dietary advice and, if treatment targets are not met additional insulin therapy. The treatment of GDM has shown to be effective in the reduction of complications (7,8). In recent years, management guidelines have been updated with more stringent diagnostic criteria from the International Association of Diabetes in Pregnancy Study Groups (IADPSG 75-g oral glucose tolerance test (OGTT): fasting glucose: ≥ 5.1mmol and 2-hour glucose: ≥ 8.5 mmol/L) (9). In the Netherlands, diagnostic criteria based on the WHO 1999 criteria (75-g OGTT fasting: ≥ 7.0 mmol/L; 2 hours: ≥ 7.8 mmol/L) are still used to date (10). The effect of this national screening and treatment guideline on pregnancy outcome has not been widely studied. Recently, Koning et al. showed that the national management guideline resulted still resulted in a higher rate of large-for-gestational-age infants compared with the general obstetric population (20% vs. 11%) (11). However, their population constituted largely of Caucasian women and thus outcomes are not applicable for all geographic areas. We investigated recent maternal and neonatal outcomes of GDM, in a large multi-ethnic cohort and compared it to the general obstetric hospital population without diabetes mellitus (GDM, Type 1 or 2 diabetes mellitus) obtained from the nationwide Dutch pregnancy registry (12). The objective of this study was 1) to analyse maternal and neonatal outcomes associated with GDM compared with the general obstetric population and 2) to determine factors predicting neonatal complications and the use of insulin therapy in a large cohort.

Population and methods

We retrospectively analysed all singleton pregnancies diagnosed with GDM in the Maasstad Hospital in Rotterdam, the Netherlands from the period 2010–2015. All data collected was obtained from electronic medical records. In the Netherlands, more than 96% of births are registered in a nationwide pregnancy registry (12). We compared our data on GDM outcomes with pregnancy outcomes from the nationwide registry. This control group (CG) (N=574.823) consisted of singleton pregnancies who had no record of diabetes mellitus type 1 & 2 or GDM in the period 2010–2014. The local medical ethics committee approved this study. For this type of study formal consent is not required.

Screening & treatment methods

Screening for GDM was performed in pregnant women with one or more of the following risk factors: BMI ≥ 30 kg/m^2, first degree family member with diabetes mellitus, previous pregnancy with GDM, history of unexplained intrauterine foetal death (IUFD), polycystic ovary syndrome (PCOS), previous infant with birth weight > 4500 gram or > 95th percentile (> P95) and certain ethnic groups; i.e. Asian, Hindu, Creole) (13). Screening was also performed in women with signs suggestive of GDM (e.g. polyhydramnios or foetal macrosomia). In summary, screening occurred in the first (random glucose level) and second trimester with a 75-g OGTT performed between 24–28 weeks of gestation. In women with a history of GDM, the OGTT was performed at 16 weeks of gestation. GDM was defined as meeting one or more of the following OGTT criteria: fasting plasma glucose ≥ 7.0 mmol/L and/or 2-hour plasma glucose ≥ 7.8 mmol/L (13,14). Treatment consisted of lifestyle and dietary recommendations. Insulin therapy was initiated if fasting and/or 2-hour postprandial capillary blood glucose levels were ≥ 5.3 mmol/L and ≥ 6.7 mmol/L respectively on two occasions at the same moment of the day. Insulin therapy consisted of either once daily long-acting insulin or (ultra)-short acting insulin or a combination of both. Insulin therapy, inadequate glycaemic control or foetal macrosomia, was an indication for induction of labour at 38 weeks of gestation. In our centre, care of women with GDM is multidisciplinary (endocrinologist, diabetes nurse, qualified dietician and obstetrician) and structured according to protocol with weekly monitoring throughout pregnancy. Patients who were referred to our centre but gave birth in another centre or at home without access to delivery data (N=44), were not included.

Outcome measures

Patient characteristics including maternal age, parity, body mass index (kg/m^2), random venous glucose levels in first trimester (mmol/L), OGTT levels (fasting and 2-hours post glucose load in mmol/L), gestational age at diagnosis and insulin therapy were recorded. The following maternal risk factors for GDM were studied: BMI ≥ 30, first degree family member with diabetes mellitus, gestational diabetes in previous pregnancy, history of unexplained IUFD, PCOS, previous neonate with birth weight > 4500 gram or (> P95). Ethnicity or racial group was self-reported and classified as Caucasian, Mediterranean, Creole, Asian, Hindu and other (13). Maternal outcome included: pregnancy-induced hypertension (PIH) (systolic blood pressure ≥ 140 and diastolic blood pressure ≥ 90 mm Hg, after 20 weeks of gestation, measured twice), preeclampsia (PIH + proteinuria of ≥ 300 mg/24 hours) and induction of labour. Insulin therapy or foetal macrosomia was an indication for labour induction at 38 weeks of gestation. Caesarean section was defined as primary (elective) or secondary (emergency). Neonatal complications were recorded to compare with the control group. Individual neonatal complications did not occur frequently therefore a composite neonatal outcome was used to determine independent predictors. The composite outcome was defined as one or more of the following: death defined as after 28 weeks of gestation or in the first seven days from birth, birth trauma (shoulder dystocia and/or brachial plexus injury and/or bone fracture (humerus/clavicle), neonatal hypoglycaemia (< 2.6 mmol/L), admission to the neonatology department, jaundice requiring phototherapy.

Statistical analysis

Results for continuous variables with normal distribution are given as mean with standard deviation (SD). In case of skewed distribution, results are presented as median with inter quartile range (IQR). Categorical variables are given as frequencies and percentages. Groups were compared by means of a Pearson's chi-squared test or Fisher's exact test. Bonferroni correction was used to correct for multiple comparisons (Table 1 & 2). To determine potential predictors of need for insulin therapy, analysis using logistic regression models was performed to determine odds ratios (ORs) and 95% confidence intervals (95% CIs). Variables included in the model were: maternal age, BMI ≥ 30, first trimester random glucose (mmol/L), 75-g OGTT: fasting glucose level (mmol/L) and 2-hours post glucose load glucose levels (mmol/L), GDM in previous pregnancy and ethnicity (Caucasian, Hindu, Creole, Asian, Mediterranean and other). Caucasian was used as the reference category. Univariate analysis was performed and significant variables were entered into a multivariable logistic regression model. *P*-values < 0.05 were considered to be significant. To examine potential predictors for the composite neonatal outcome, univariate logistic regression analysis was performed to determine the odds ratio's (ORs) and 95% confidence intervals (95% CIs). Variables included were: maternal age, BMI ≥ 30, random glucose level in first trimester (mmol/L), 75-g OGTT: fasting and 2 hour post glucose load glucose levels (mmol/L), GDM in a previous pregnancy, insulin therapy and ethnicity (Caucasian, Hindu, Creole, Asian, Mediterranean and other). Caucasian was used as the reference category. Variables with $P < 0.05$ were entered into a multivariate regression model to test for independence when multiple positively correlated factors were found. Statistical analysis was performed using SPSS Statistics version 23 (IBM Corp, Armonk, NY, USA).

Results

A total of 1008 consecutive singleton pregnancies were analysed. Population characteristics are shown in (Table 1). In GDM, the majority of women were overweight (BMI ≥ 25=34.2%) or obese (BMI ≥ 30=33.9%) in the first trimester of pregnancy. The most frequent risk factors for GDM in our population observed were: BMI ≥ 30 (33.9%), first degree family member with diabetes mellitus (42.2%). Additional insulin therapy was required in (38.7%) of our population. Our population is multi-ethnic with 43.5% Caucasian vs. 73.4% in the CG. Maternal and neonatal outcomes are shown in (Table 2). After Bonferroni correction, significant differences were observed for age, parity, ethnicity, Apgar score and brachial plexus injury. Differences were small and may not be of clinical relevance (Table 1 & 2). A significant difference was observed for the rate of labour induction, which was about two times higher in our population versus CG (56% vs. 29.2%) ($P < 0.001$). Independent predictors for the need of insulin therapy were: BMI ≥ 30, OGTT glucose levels: (fasting and 2-hours) and GDM in a previous pregnancy and ethnicity (Mediterranean) (all $P < 0.05$) (Table 3). In the univariate analysis with the neonatal composite outcome as the dependent variable, the following parameters were positively correlated: maternal age, BMI ≥ 30, random first trimester glucose level (mmol/L), OGTT: fasting and 2 hours glucose level (mmol/L), insulin therapy, GDM in a previous pregnancy (all $P < 0.05$). Subsequently the multivariate analysis showed that BMI ≥ 30 (OR 1.6 [1.1–2.3] P=0.041) and insulin therapy (OR 2.5 [1.7–3.9] P=0.001) were significant predictors (Table 4).

Table 1 | Population characteristics

Parameters	GD (N=1008)	CG (N=574.823)	*P*-value*
Age (yr)			0.001††
< 20	9 (0.9)	8769 (1.5)	
20–25	102 (10.1)	62915 (10.9)	
25–30	280 (27.8)	175459 (30.5)	
30–35	340 (33.7)	206220 (35.9)	
35–40	211 (20.9)	100608 (17.5)	
> 40	66 (6.5)	20845 (3.6)	
Parity (N)			0.001††
Nulliparous	247 (24.5)	296754 (51.6)	
Multiparous	761 (75.5)	278069 (48.4)	
BMI (kg/m^2) in first trimester	28.2 ± 6.1	N/A	
< 18.5	15 (1.5)		
18.5–24.9	306 (30.4)		
25–29.9	345 (34.2)		
30–34.9	200 (19.8)		
> 35	142 (14.1)		
Risk factors for GD		N/A	
First degree family member with diabetes mellitus	420 (42.2)		
Unexplained history of intra-uterine foetal death	38 (3.8)		
Previous child with birth weight > 4500 gram or > p95	70 (6.9)		
Previous pregnancy with gestational diabetes	112 (11.1)		
Polycystic ovary syndrome	24 (2.4)		
BMI ≥ 30	342 (33.9)		
Race or ethnic group			
Caucasian	438 (43.5)	422062 (73.4)	0.000††
Mediterranean	252 (25)	39552 (6.9)	
Creole	121 (12)	17387 (3.0)	
Hindu	92 (9.1)	7556 (1.3)	
Asian	36 (3.6)	15670 (2.7)	
Other	44 (4.4)	70850 (12.3)	
Unknown	25 (2.5)	1746 (0.3)	
Random glucose level first trimester – mmol/L	5.2 ± 1.2	N/A	
75-g OGTT: fasting glucose level – mmol/L	5.4 ± 1.2	N/A	
75-g OGTT: 2 hour glucose level – mmol/L	9.3 ± 1.7	N/A	
Gestational age at diagnosis: gestational diabetes – weeks	28 [24–32]	N/A	
Insulin therapy	389 (38.7)	N/A	
Gestational age at start of insulin therapy – weeks	32 [27–35]	N/A	

Data presented as mean ± SD, frequency (%) or median [IQR]; **P*-value < 0.05 was considered statistical significant tested with Pearson's Chi-squared test; † Statistically significant after Bonferroni correction ($p < 0.05/3$); N/A: data not available.

Table 2 | Maternal and Neonatal Outcomes

Parameters	GD (N=1008)	CG (N=574.823)	*P*-value*
Maternal outcomes			
Pregnancy-induced Hypertension	49 (4.9)	N/A	
Pre-eclampsia	38 (3.8)	29027 (5.1)	0.064
Labour induction	564 (56)	168079 (29.2)	0.000†
Labour induction due to gestational diabetes¥	370 (65.5)	N/A	
Caesarean section	242 (24)	126770 (22.1)	0.135
Primary	118 (48.8)	56809 (44.8)	
Secondary	124 (51.2)	69961 (55.2)	
Neonatal Outcomes			
Pre-term birth (< 37 weeks)	67 (6.6)	46826 (8.1)	0.082
Apgar < 7 at 5 minutes	88 (8.7)	14895 (2.6)	0.000†
Birth weight (gram)			0.003
< 3000	252 (25.0)	124065 (21.6)	
3000 – < 4000	648 (64.3)	371582 (64.6)	
> 4000	108 (10.7)	78518 (13.7)	
Congenital anomaly	22 (2.2)	21604 (3.8)	0.009
Perinatal death	8 (0.8)	4140 (0.7)	0.783
Large for gestational age (> P90)	86 (8.5)	61917 (10.7)	0.022
Neonatal hypoglycaemia (< 2.6 mmol/L)	160 (15.9)	N/A	
Admission to neonatology department	219 (21.7)	N/A	
Neonatal hypoglycaemia requiring intravenous glucose	94 (58)	N/A	
Hyperbilirubinemia requiring phototherapy	29 (2.9)	N/A	
Birth trauma			
shoulder dystocia	19 (1.9)	8278 (1.4)	0.237
clavicle fracture	4 (0.4)	5889 (1.0)	0.048‡
brachial plexus injury	2 (0.2)	29 (0.01)	0.001‡†

Data presented as frequency (%); **P*-value < 0.05 was considered statistical significant tested with Pearson's Chi-squared test; † Statistically significant after Bonferroni correction ($p < 0.05/12$); ‡ For clavicle fracture and brachial plexus injury Fisher's exact test was used; ¥ Labour induction due to gestational diabetes was performed in those using insulin therapy; N/A: data not available.

Table 3 | Multivariable logistic regression analysis of predictors for additional insulin therapy

Parameters	OR	95% CI	*P*-value*
BMI ≥ 30 (kg/m^2)	1.5	1.1–2.2	0.014
75-g OGTT: fasting glucose level – mmol/L	1.2	1.1–1.4	0.009
75-g OGTT: 2 hour glucose level – mmol/L	1.3	1.2–1.4	0.000
Previous pregnancy with gestational diabetes	2.9	1.4–5.0	0.000
Race/ethnicity group: ‡			
Mediterranean	1.5	1.1–2.0	0.048

OR, 95% confidence intervals and *P*-values were derived from a logistic regression model; **P*-value < 0.05 was considered statistical significant; ‡ Caucasian was used as the reference group.

Table 4 | Multivariate regression analysis of predictors for composite neonatal outcome

Parameters	OR	95% CI	*P*-value*
Age (y)	0.9	0.9–1.0	0.827
BMI > 30 (kg/m^2)	1.6	1.1–2.3	0.041
Random glucose levels first trimester – mmol/L	1.2	1.0–1.4	0.032
75-g OGTT: fasting glucose level – mmol/L	0.8	0.7–1.0	0.156
75-g OGTT: 2 hour glucose level – mmol/L	1.0	0.9–1.1	0.433
Gestational diabetes in previous pregnancy	1.4	0.7–2.6	0.326
Insulin therapy	2.5	1.7–3.9	0.001

OR, 95% confidence intervals and *P*-values were derived from a logistic regression model; **P*-value < 0.05 was considered statistical significant.

Discussion

In our single centre Rotterdam cohort, with GDM screening based on risk factors, adverse maternal and neonatal outcomes in GDM were similar to the general obstetric population without diabetes mellitus. This indicates that in women diagnosed with 'higher' levels of hyperglycaemia compared to the current international diagnostic criteria (IADPSG); a low rate of adverse pregnancy outcomes can be achieved. In the Netherlands, healthcare is easily accessible irrespective of socio-economic background. In our centre, care for women with GDM is multidisciplinary and structured according to protocol with weekly monitoring throughout pregnancy. Furthermore, initiation of insulin therapy is based on strict criteria. Perhaps these factors contributed to a well-controlled GDM cohort. Two landmark studies in the treatment of GDM have shown that treatment reduces adverse perinatal outcomes (7,8). Crowther et al. reported higher rates of preeclampsia, caesarean delivery and LGA and lower rates of labour induction in the treatment group (7). Landon et al. showed similar rates of LGA, caesarean delivery and shoulder dystocia in the treatment group (8). However, it must be noted that these studies used different diagnostic criteria and treatment targets. Koning et al. studied GDM

pregnancies using the same screening and treatment guideline but reported a higher rate of LGA infants. However, women in their cohort were more obese and different postprandial treatment targets were used. Perhaps the high rate of labour induction at 38 weeks of gestation in combination with strict management policy prevented a high rate of LGA infants in our population. Data has shown that elective induction of labour in suspected LGA reduces the risk of shoulder dystocia and does not increase the risk of caesarean section (15,16).

BMI ≥ 30, OGTT glucose levels: (fasting and 2-hours post glucose load), GDM in a previous pregnancy and ethnicity (Mediterranean) (all $P < 0.05$) were independent predictors for the additional use of insulin therapy. OGTT glucose levels are measures of hyperglycaemia and related to defects in insulin sensitivity and secretion (17). Other studies have also demonstrated that ethnicity, gestation at diagnosis, HbA_{1c}, OGTT levels, BMI, and diabetes family history are also significant independent determinants of insulin therapy (18,19). In GDM, insulin therapy and obesity were independently associated with the composite neonatal outcome.

Obesity was the second most prevalent risk factor and independently associated with the composite neonatal outcome. The prevalence of obesity in Dutch women between 18–29 years old is estimated at 7.6% and between 30–49 years old 15.6% (20). More than a third of our study population was obese in the first trimester of pregnancy. Obesity is associated with increased risk of significant adverse maternal and neonatal outcomes (21). Meta-analysis showed an unadjusted OR for developing GDM of 2.14 (95% CI 1.82–5.53), 3.56 (3.05–4.21), and 8.56 (5.07–16.04) in overweight, obese and severely obese respectively compared to normal weight pregnant women (22). Data from the HAPO study showed that the rate of macrosomia was twice as high in GDM compared to women with normal glucose tolerance, in women with and without obesity. Macrosomia in GDM was seen in 26%, in GDM + obesity in 33% and in obesity only in 41% (23). Another study demonstrated that maternal obesity is a stronger predictor of a large for gestational age infant than maternal hyperglycaemia (24). A large prospective study showed that the upper quartile of pre-pregnancy BMI was responsible for 23% of macrosomia, while GDM accounted for only 3.8% (25). Apparently, obesity and GDM must be considered as independent predictors of macrosomia. Strengths of this study include: large cohort of multi-ethnic women, in the background of a similar CG in terms of time period and obstetric setting. Limitations of this study include: retrospective design and the relatively out-dated WHO 1999 diagnostic criteria (14). Furthermore, pre-pregnancy BMI and gestational weight gain (GWG) was not available. For the CG, data on GDM risk factors, BMI and OGTT levels was not available. Therefore, women with glucose intolerance could potentially have been included.

Finally, we suggest that before adopting the stricter IADPSG criteria, well-designed intervention trials showing treatment and cost-benefit must be performed. As previously shown treatment benefits cannot be automatically assumed for this new GDM population.

Conclusions

In conclusion, we observed in a large multi-ethnic cohort, that when GDM is treated according to the current national guideline, a low rate of adverse pregnancy outcomes is achieved. Obesity and insulin therapy seem to be predictive of an increased risk of a neonatal complication in women with GDM. Our study underlines the importance of a proper weight control before pregnancy. Health care professionals should play an important role in the prevention of obesity in young women.

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CHAPTER 5

Comparison of SMBG and CGM in Gestational Diabetes Mellitus

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Submitted for publication

Abstract

Aims: Monitoring of treatment in women with gestational diabetes mellitus (GDM) is based on self-monitoring of blood glucose (SMBG). The aim was to determine the percentage of time in hyperglycaemia with blinded continuous glucose monitoring (CGM) in women with GDM and a normal SMBG profile.

Methods: Twenty-seven women with GDM underwent a 72-hour monitoring period with blinded CGM after week 20 of gestation. SMBG was performed (7 times) and compared with CGM on day 2. Hyperglycaemia in SMBG and CGM was defined as ≥ 5.3 mmol/L fasting and/or ≥ 6.7 mmol/L 2-hour postprandial. Parameters of glycaemic variability were reported for the 72-hour monitoring period.

Results: Of the 27 women in this study, 17 women had no hyperglycaemia according to the 7-point SMBG profile performed on day 2. In these 17 women, blinded CGM revealed hyperglycaemia in three women (17.6 %), which was not detected by SMBG. The percentage of time in hyperglycaemia was 13.8% on day 2. The percentage of time of time in target range (4.0–6.6 mmol/L) during the 72-hour period was 79.3%. The mean amplitude of glycaemic excursions (MAGE) was 2.0 ± 0.7 mmol/L, while the Mean of Daily Differences (MODD) was 1.0 ± 0.4 mmol/L, and the interquartile range (IQR) was 1.3.

Conclusion: Blinded CGM allowed for the detection of hyperglycaemia that had been missed by the 7-point SMBG profile in 17.6% of GDM women in our study. However, the additional diagnostic value in a well-controlled GDM population was limited.

Introduction

Gestational diabetes mellitus (GDM) is a frequent complication of pregnancies and is associated with maternal and perinatal morbidity (1). Treatment consists of dietary and lifestyle intervention and if glycaemic targets are not met, insulin therapy is initiated. Two landmark studies showed that treatment leads to a reduction in perinatal complications (2,3). The goal of treatment is to reach adequate glucose levels and therefore accurate insight into glucose regulation is necessary.
Currently, monitoring of GDM treatment is based on self-monitoring of blood glucose levels (SMBG) by means of finger-stick glucose measurements. Postprandial hyperglycaemia is the hallmark of GDM and subsequently glucose measurements are performed 1- to 2-hour after a meal. SMBG can lower the rate of foetal overgrowth in women with mild GDM (4). The optimal frequency and timing of glucose measurements is unknown and compliance seems to be low (5).
Ideally, insight into a longitudinal glucose profile should be available as especially the glucose variability appears to be associated with the onset of diabetic complications (6,7).
Continuous glucose monitoring (CGM) is a glucose monitoring technique providing insight into glucose fluctuations over the day including postprandial glucose peaks, as well as periods of hyper-and hypoglycaemia (8). CGM appears to be safe and effective in assessing glycaemic profiles in patients with and without diabetes (5,9,10). Recently, the additional clinical benefit of CGM in pregnant women with type 1 diabetes was shown by Feig et al. (11). The aim of our study was to evaluate the potential of blinded CGM for the detection of hyperglycaemia in women with GDM. We, therefore, studied the percentage of time in hyperglycaemia detected by blinded CGM when SMBG showed no hyperglycaemia in a 24 hour period. Secondly, we studied measures of glycaemic variability.

Methods & materials

Subjects

This observational study was performed by the diabetes outpatient clinic of the Maasstad Hospital, the Netherlands. Pregnant women were screened and treated for GDM according to the national guideline by the Dutch Society of Obstetrics and Gynaecology (12). Screening was performed if women had one or more of the following risk factors: previous pregnancy with GDM, first-degree relative with diabetes mellitus (DM), a previous neonate with birth weight ≥ 4500 gram or > 95th percentile, pre-pregnancy body mass index (BMI) > 30 kg/m^2, some ethnic risk groups (South-Asian, Hindu, African-Caribbean, Middle Eastern, Morocco and Egypt), history of intrauterine foetal death (IUFD), and history of polycystic ovary syndrome (PCOS). Screening was performed by means of a 75-g oral glucose tolerance test (OGTT) between 24–28 weeks of gestation. GDM was diagnosed if the fasting plasma glucose was ≥ 7.0 mmol/l and/or the two-hour plasma glucose ≥ 7.8 mmol/l. Women with the diagnosis of GDM, ≥ 18 years old, and singleton pregnancy judged by ultrasonography were eligible for inclusion. All subjects received treatment for GDM according to standard care. Treatment consisted of lifestyle and dietary advice by a trained dietician and counselling by a diabetologist.

Women monitored their glucose 7 times (pre- and 2-hour postprandial and before sleeping) a day, with finger-stick glucose measurements using a OneTouchSelect Plus (Johnson and Johnson Medical, the Netherlands). Treatment targets were fasting < 5.3 mmol/L and < 6.7 mmol/L 2-hour postprandial (12). Insulin therapy was initiated if treatment targets were not met within one week. The local medical ethics committee approved this study. All subjects gave written informed consent.

Continuous glucose monitoring

Blinded CGM measures glucose levels through electro-chemical detection in the extracellular fluid of the subcutaneous tissue and records values in a range of 2.2–22.2 mmol/L every 10 sec. A mean value is calculated and stored by the device every 5 minutes, providing up to 288 glucose measurements a day. We used blinded CGM which does not display glucose values; data are stored in the device and uploaded to a computer after removing the sensor. All women received blinded CGM (iPro™2 Medtronic, Northridge, CA, USA) regardless of GDM treatment (diet or with additional insulin therapy). Blinded CGM monitoring was initiated after week 20 of gestation. Women came to the outpatient clinic and the CGMS was implanted in the flank and affixed by Tegaderm® plaster. The monitoring periods consisted of 72 consecutive hours. During the monitoring periods women were instructed to perform SMBG with finger-stick glucose measurements (day 1: 3 measurements for calibration; day 2: 7 measurements (standard care); and day 3: 3 measurements for calibration). If women were on insulin therapy, then 7 finger-stick glucose measurements per day were performed during the monitoring period. Women were instructed to record breakfast, lunch and dinner time in a diary. After 72 hours, blinded CGM sensors were removed and data were uploaded into Carelink™ iPro Therapy management Software for Diabetes (Medtronic, Northridge, CA, USA). The CGM profiles were calibrated with 3 SMBG. If the iPro™2 recorded less than 36 hours of data (< 50%) in a patient due to sensor malfunctioning, then she was excluded from the study. CGM data analysis was performed after all women completed the study.

Glycaemic parameters

The aim was to determine the percentage of time in hyperglycaemia detected by blinded CGM in women with a normal 7-point SMBG profile on day 2 of the monitoring period. The percentage of time in hyperglycaemia was calculated from 18 hours on day 2 (24 hours – (3 x 2-hours postprandial time)=18 hours). A normal SMBG profile was defined as fasting < 5.3 mmol/L and/or < 6.7 mmol/L 2-hours postprandial, in line with treatment targets. Hyperglycaemia in CGM was defined as ≥ 5.3 mmol/L fasting (defined as the mean of six consecutive values (30 min) starting at 0600 a.m.) and/or ≥ 6.7 mmol/L 2-hour postprandial (13). For the other glycaemic parameters the 72-hour monitoring period was used. Parameters included: time in target range 4.0–6.6 mmol/L (based on treatment target < 6.7 mmol/L), the percentage of time in the hyperglycaemia range (≥ 6.7 mmol/L), the percentage of time in the hypoglycaemic range (< 3.9 mmol/L), the mean blood glucose (MBG), the standard deviation (SDBG), mean amplitude of glycaemic excursions (MAGE), Mean of Daily Differences (MODD), and interquartile range (IQR). The MAGE was calculated by measuring the mean of glycaemic oscillations exceeding the standard deviation of the sensor glucose measurement. The MAGE is a parameter designed to quantify major glycaemic patterns, ignoring minor swings, and

was used for assessing intra-day glycaemic variability. The MODD is the mean of absolute values of differences between glucose values taken in two consecutive days. CGM-derived variables were calculated using the GlyVaRT program, version 1.2.1 (Medtronic Bakken Research Centre, Maastricht, The Netherlands).

Statistical analysis

Data are presented as mean ± SD for continuous variables with normal distribution or as median values with IQRs (25^{th}–75^{th} quartiles) in case of a skewed distribution. Categorical data are shown as frequency and percentages. SPSS release 16.0 (IBM Corp.; NY; USA) was used for data analyses.

Results

In total, 31 pregnant women with GDM participated in this study between 2015–2017. Four women were excluded due to sensor malfunctioning (recording time < 50%). Two of the excluded women had hyperglycaemia shown by SMBG. Patient characteristics are shown in (Table 1).

Table 1 | Patient characteristics

Parameters	N=27
Age (years)	32.5 ± 6
Parity	1 [0-2]
BMI (kg/m^2)	28 ± 5
Gestational age at diagnosis – weeks	24 ± 5.5
75-g OGTT: fasting glucose – mmol/L	5.3 ± 0.6
75-gOGTT: 2 hour postprandial glucose – mmol/L	9.3 ± 1.2
Insulin therapy (%)	6 (22.2)

BMI: Body Mass Index; OGTT: oral glucose tolerance test
Data presented as mean with SD, median with interquartile range or frequency with percentage.

Women tolerated blinded CGM well and all subjects completed the study. No local sensor insertion site adverse events were observed.
Of the 27 women in this study, 17 women had no hyperglycaemia according to the 7-point SMBG profile performed on day 2. In these 17 women, blinded CGM revealed hyperglycaemia in three women (17.6 %), which was not detected by SMBG. The percentage of time in hyperglycaemia detected by blinded CGM in women with a normal 7-point SMBG profile on day 2 was 13.8%. One woman showed a mild fasting hyperglycaemic episode (mean glucose of 5.4 mmol/L during 30 minutes). One woman had 1 hour of hyperglycaemia (before lunch and evening), and one woman had 20 minutes of hyperglycaemia which persisted after the 2-hour postprandial measurement.

The other glycaemic variability parameters were determined in the total population (N=27) and are shown in Table 2. Using blinded CGM, a mean number of 882 ± 215.8 glucose measurements was recorded for each woman. The percentage of time of glucose levels within the normal range (4.0–6.6 mmol/L) was 79.3%. The percentage of time within the hyperglycaemic range (≥ 5.3 fasting and ≥ 6.7 mmol/L 2-hour postprandial) was 8.4%. The percentage of time within the hypoglycaemic range (< 3.9 mmol/L) was 9.1%. Of the 27 women in this study, 18 women had no hypoglycaemia according to the 7-point SMBG profile performed on day 2. In these 18 women, blinded CGM revealed hypoglycaemia on day 2 in 5 women (27.7%) that was not detected by SMBG. The MAGE (mmol/L) was 2.0 ± 0.7, the MODD (mmol/L) was 1.0 ± 0.4 and the IQR was 1.3.

Table 2 | Glycaemic parameters from 72-hour CGM period

Parameters	N=27
Mean total points (N)	882 ± 215.8
Mean blood glucose (MBG) (mmol/L)	5.2 ± 0.5
Standard deviation of blood glucose (SDBG)	1.0 ± 0.3
Percentage of time in target (4–6.6 mmol/L) (%)	79.3
Percentage of time in hyperglycaemia (≥ 6.7 mmol/L) (%)	8.4
Percentage of time in hypoglycaemia (< 3.9 mmol/L) (%)	9.1
Mean amplitude of glucose excursion (MAGE) (mmol/L)	2.0 ± 0.7
Mean of Daily Difference (MODD) (mmol/L)	1.0 ± 0.4
Interquartile range (IQR) (mmol/L)	1.3

Discussion

In this small observational study we showed that blinded CGM detected hyperglycaemia in 17.6% of the women, which was not detected by 7-point SMBG profile. Although, blinded CGM identified hyperglycaemia in three additional women, the percentage of time in hyperglycaemia was not circumstantial. This could imply that current GDM management strategies are adequate. Furthermore, even similar average glucose levels were found in studies evaluating daily glucose profiles with CGM in healthy non-diabetic pregnant women (14,15). However, due to study design we were not able to evaluate the effect of CGM on pregnancy outcome.

Of the women who were identified with hyperglycaemia by blinded CGM, two out of three were managed with diet and lifestyle advice. In these cases the diagnostic value of blinded CGM might have translated into a treatment intervention with insulin therapy. In a study by Kestila et al., authors showed that blinded CGM detected a higher proportion of women with GDM requiring anti-diabetic treatment compared with SMBG (16). McLachlan et al. showed that blinded CGM revealed additional information in patients with type 1, type 2 diabetes and GDM, which was not shown by SMBG. This

altered clinical decision-making in a majority of cases but was most useful in patients with type 1 diabetes (17).

Women might adhere better to diet and lifestyle recommendations on days that SMBG is required. Increasing SMBG frequency could have a possible negative effect on compliance. Moreover, the optimal frequency of SMBG to minimize adverse pregnancy outcome is unknown. In our study, one woman had hyperglycaemia in the period before lunch and in the evening. The other woman had a short period of hyperglycaemia (20 minutes) after the postprandial measurement. Therefore, blinded CGM could identify glucose patterns especially during periods which are not registered by SMBG. Furthermore, it could serve as an additional evaluation tool, assess treatment compliance, and help optimize therapeutic recommendations (18). Recent data from the recent CONCEPTT trial showed improved obstetric and neonatal outcome (reduced large for gestational age, shorter duration of stay in neonatal intensive care unit, and less neonatal hypoglycaemia requiring treatment) with real-time CGM in women with type 1 diabetes and this will likely change current practice (11).

In our study, SMBG was performed 2 hours after meals with adequate detection of hyperglycaemia in most cases. Bühling and colleagues reported that the optimal timing of postprandial glucose values appears to be between 45 and 120 minutes in a study that included 13 GDM and 4 type 1 diabetic pregnant women (19). Ben-haroush et al. showed that the postprandial glucose peak in diabetic pregnancies is approximately 90 minutes after meals (20). Chen et al. demonstrated that CGM detected periods of hyperglycaemia and hypoglycaemia that were not shown by SMBG (9). However, the composition of the meal affects the highest peak value and may be missed by a single postprandial measurement.

The percentage of time in hypoglycaemia was slightly higher than the percentage of time spent in hyperglycaemia (9.1% vs. 8.4%, respectively). In five women, blinded CGM revealed time spent in hypoglycaemia that was not detected by SMBG. Nevertheless, it is probably within the physiological range as none of these women were treated with insulin therapy (15). In general, the effects of hypoglycaemia on perinatal outcome have been studied less often, but animal studies show possible risk of congenital abnormalities, even with short durations of hypoglycaemia (21). Data has shown that CGM could aid in the evaluation of asymptomatic hypoglycaemic episodes (22,23).

Glycaemic variability probably has more harmful effects than sustained chronic hyperglycaemia in the development of diabetic complications (24). In this study, MBG, SDBG, MAGE, and MODD were used to analyse glycaemic variability. Yu et al. showed similar results for MAGE and MODD (25). They showed that glycaemic variability and birth weight of neonates of women with GDM were improved by means of CGM compared with SMBG. Furthermore, they showed that MAGE was an independent predictor of birth weight. Darlfra et al. suggested that although measures of glycaemic variability were only slightly higher in GDM than in controls, it could still interfere with foetal growth (26). The effect of glycaemic variability in GDM patients requires further investigation. A limitation of our study was that the sample size was small and the monitoring period brief. If longer and more frequent blinded CGM periods were performed, then data might have been more conclusive. In general, a number of methodological issues need to be addressed to advance both blind and real-time CGM research. First of all, uniformity in CGM parameter definitions is required to be able to

compare data (13). Secondly, the duration of CGM during pregnancy needs to be long enough to study the effect on pregnancy outcome (27).
In conclusion, we observed a well-controlled GDM cohort under the current screening and treatment guideline. Blinded CGM improved the detection of hyperglycaemia slightly compared with SMBG but the additional diagnostic benefit remains small for this population. Due to study design and sample size, the clinical value of CGM versus SMBG, for improving pregnancy outcome could not be assessed.

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Conflicts of Interest

None declared.

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CHAPTER 6

Metformin in women with high risk of Gestational Diabetes Mellitus

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Abstract

Aim: To study the effect of metformin on the incidence of gestational diabetes mellitus (GDM) in pregnant women with a high risk of GDM.

Methods: In this open-label randomized-controlled trial we randomly assigned 49 pregnant women with a high risk of GDM to receive metformin (N=24) at a dose of 2.0 g per day, or no intervention (N=25) from 14 weeks of gestation until delivery. The primary endpoint was the incidence of GDM in both groups. The secondary endpoints included maternal and neonatal outcomes.

Results: A total of eight women withdrew consent during the trial, which left 18 women in the metformin group and 23 in the control group. There were no significant between-group differences at baseline. We could not detect significant between-group differences in the incidence of GDM, large-for-gestational-age infants, or adverse maternal or neonatal outcomes. The rate of elective labour induction was higher in the control group than in the metformin group (72.7% vs. 38.9%, *P*=0.031).

Conclusions: Metformin treatment of pregnant women from 14 weeks until delivery appears to have no influence on the incidence of GDM is this small population. No harmful effects of metformin use in pregnancy were observed. Long-term follow-up of children exposed to metformin in pregnancy is important for recruitment of women in clinical trials and in clinical practice.

Introduction

The prevalence of gestational diabetes mellitus (GDM) is increasing worldwide (1). Attempts at reducing adverse pregnancy outcome through dietary and lifestyle interventions have shown modest effects, with no significant impact on GDM incidence and related outcomes (2-4).
Metformin, a biguanide analogue, increases insulin sensitivity and decreases hepatic glucose production (5). Therefore, metformin might also reduce insulin resistance in pregnancy, leading to less maternal weight gain, a reduction in maternal glucose levels and subsequent foetal hyperinsulinaemia. In turn, this could have a positive effect on neonatal birth weight and the risk of obesity and diabetes later in life. Evidence thus far supports the safety and efficacy of the use of metformin during pregnancy, but there is still disparity in the acceptance of it as long-term outcome in children is unknown (6-9).
In women with polycystic ovary syndrome (PCOS) who used metformin in early pregnancy, reports on the effect on the incidence of GDM have been conflicting (10,11).
To date, two large double-blind randomized-controlled trials (The EMPOWaR and The MOP Trial) showed no significant effect of metformin on birth weight percentile in obese pregnant women (12, 13). We investigated the effect of metformin, not only in obese pregnant women, but also in women with other risk factors for GDM.

Methods

In this open-label randomized controlled trial, we randomly assigned women with a high risk to develop GDM to receive either metformin at 14 weeks of gestation or no intervention (control group). High risk was defined according to the Netherlands Society of Obstetrics and Gynaecology guideline and included: GDM in a previous pregnancy, body mass index > 30 (kg/m^2) at the first prenatal screening, birth weight previous child > 4500 gram or > 95th percentile, first degree relative with diabetes mellitus, certain ethnic groups with a high prevalence of diabetes (e.g. Black, Asian, Hindu), history of unexplained intra-uterine foetal death, and history polycystic ovary syndrome (PCOS) (14). Eligible women were between 18 and 41 years old and were less than 14 weeks pregnant. Exclusion criteria included pre-gestational diabetes, no singleton pregnancy judged by ultrasonography, known cardiac insufficiency, known renal- and liver-disease. The local ethics committee NL48005.101.14 approved this study. All participants provided written informed consent. Clinical Trials.gov number NCT02275845.

Randomization & intervention

Eligible women were randomly assigned, in a 1:1 ratio, with the use of a computer-generated randomization, to either the metformin- or the control group. Metformin was started at 14 weeks of gestation (500 mg per day in the first week and 1000 mg per day from the start of the second week and continued until delivery). Women with adverse events (AE), possibly related to the use of high-dose metformin were asked to take metformin in their maximal individual tolerated dose.

All subjects received a standard diet of 2000 calories per day with an adequate distribution of carbohydrates during the day, as recommended by the Netherlands Diabetes Federation (15).

Procedures

At enrolment (< 14 weeks of gestation), blood samples were taken after an overnight fast to asses baseline hyperglycaemia and renal- and liver-function. We excluded patients with impaired renal function (urea > 6.6mmol/L, creatinine > 85 µmol/L), or liver function (bilirubin > 16 µmol, alanine transferase > 60 µmol, or high fasting glucose in first trimester (> 6.1 mmol/L). Maternal BMI (kg/m^2) was recorded at enrolment.

Follow-up visits were scheduled at 16 (in case of previous GDM), 24, 28, and 32 weeks of gestation. During each visit maternal weight, blood pressure and adherence to treatment was recorded. Lack of adherence to treatment was defined as not taking metformin 1–3 times a week, 4–6 times a week, or > 6 times a week. All women underwent a 75-g oral glucose tolerance test (OGTT) at 24 weeks of gestation (or at 16 weeks in case of previous GDM); metformin was not taken on the day of the OGTT. Women with normal OGTT results continued with the study procedures. GDM was diagnosed based when one or more plasma glucose level was elevated (fasting plasma glucose ≥ 7.0 mmol/L and/or 2-hour plasma glucose ≥ 7.8 mmol/L) (16). Women diagnosed with GDM were advised to continue on the assigned study group. In addition, they received insulin therapy if treatment targets (fasting < 5.3 mmol/L; 2-hour postprandial < 6.7 mmol/L) were not met within one week. In accordance with standard protocol elective labour induction was performed at 38 weeks of gestation in women requiring insulin therapy.

Outcome measures

Patient demographics and clinical data were recorded at enrolment (< 14 weeks of gestation).

The primary outcome was the incidence of GDM in both groups. Other maternal outcomes included gestational weight gain (which was defined by the difference in weight at enrolment and the last antenatal visit), insulin therapy, pregnancy-induced hypertension (PIH) (systolic blood pressure ≥ 140 and diastolic blood pressure ≥ 90 mm Hg after 20 weeks of gestation, measured twice), preeclampsia (PIH + proteinuria of ≥ 300 mg/24 hours), and delivery by cesarean section (primary or secondary). Neonatal outcomes included intra-uterine foetal death (any foetal death before onset of labor or no signs of life in utero after 20 weeks of gestation), early pregnancy loss (< 20 weeks), gestational age at birth (weeks), birth weight (g), head circumference at birth (cm), length at birth (cm), pre-term birth (< 37 weeks), large for gestational age > 90th percentile (adjusted for parity), birth trauma (clavicle fracture and/or shoulder dystocia and/or brachial plexus injury), respiratory distress (respiratory support or supplemental oxygen), admission to neonatal intensive care unit (NICU), low 5-minutes Apgar score (< 7), neonatal hypoglycaemia (< 2.6 mmol/L), and hyperbilirubinaemia requiring phototherapy.

Adverse events

Patients were instructed to contact the investigator in case of an AE. In that case, the time of onset, severity, treatment required and relation to the study treatment were recorded. A data safety monitoring board oversaw the study. All serious AEs were reported to the data safety monitoring board.

Statistical analysis

Sample size calculation

We estimated that 176 patients in each group would provide 80% power to detect a 50% reduction of GDM in the metformin group. The significance level of the test was targeted at α=.05. After allowing for an expected withdrawal of 10%, we calculated that we would need to recruit 400 patients (200 per group). The primary endpoint (GDM incidence in both groups) was assessed by means of a chi-square test. Comparisons between groups were performed with the independent T-sample test or Mann-Whitney U test. Categorical variables were tested with the use of chi-square or Fisher's exact when the event counts were small.

Results

Study population

The study period was from October 2014 till February 2017. A total of 51 women with a high risk for GDM agreed to participate. Two women were excluded due to fasting glucose level > 6.1 mmol/L. A total of 49 women were included, 24 women allocated to the metformin group and 25 women to the control group. Unfortunately, 6 women in the metformin group were lost to follow up (two before start of metformin, three prior to 24 weeks of gestation, and one at 28 weeks of gestation). One subject in the control group was lost to follow up immediately after randomization. In addition, one subject in the control group had an early pregnancy loss. Maternal characteristics are shown in Table 1. There were no significant differences at baseline between the metformin group and the control group. The maximum tolerated daily dose of 2 g metformin was reached in 21 (87.5%) of 24 women. Two subjects stopped metformin and in another subject the dose was lowered to 500mg bid, due to gastro-intestinal side effects. Metformin adherence was good in 14 (77.8%) women. Three subjects did not take metformin 1–3 times a week, none 4-6 times a week, and one subject forgot to take metformin more than 6 times a week.

Table 1 | Maternal characteristics and risk factors for GDM in early pregnancy

Characteristic	Metformin Group N=24	Control Group N=25	*P*-value*
Maternal age – (yr)	29.3 ± 5.2	30.7 ± 5.2	0.818
Maternal weight (kg) < 14 weeks gestation – kg	87.0 ± 18.0	81.5 ± 16.2	0.744
Body mass index – kg/m^2	31.3 ± 5.8	30.0 ± 5.5	0.792
Length of gestation at enrollment – wk	11.7 ± 1.2	12.3 ± 1.4	0.500
Race or ethnic group[a]			
White	9 (37,5)	8 (32)	0.686
Black	6 (25)	9 (36)	0.404
Moroccan	4 (16,7)	3 (12)	0.476
Asian	1 (4,2)	0 (0)	0.490
Hindu	0 (0)	1 (4)	0.510
Other[b]	3 (12,5)	3 (12)	0.646
Risk factors for GDM – no. (%)			
GDM in previous pregnancy	5 (20.8)	10 (40)	0.146
BMI > 30 at first prenatal screening	14 (58.3)	13 (52)	0.656
Previous infant with birth weight > 4500 gram or > 95th percentile	0(0)	1(4)	0.322
First degree family member with diabetes mellitus	11 (45.8)	16 (64)	0.201
History of polycystic ovary syndrome	1 (4.2)	1 (4)	0.976
History of unexplained intra-uterine foetal death	1 (4.2)	1 (4)	0.976
75-g OGTT: fasting glucose – mmol/L	5.6 ± 0.8	5.6 ± 0.8	0.896
75-g OGTT: 2 hour glucose – mmol/L	8.2 ± 1.8	8.4 ± 1.6	0.646

Data presented as mean ± SD or frequency (%); [a]Race or ethnic group was self-reported; *P*-value < 0.05 was significant

Outcome measures

The incidence of GDM did not differ between the metformin group and the control group (10 (55.6%) vs. 16 (69.6%), *P*=0.355) (Table 2). The incidences of maternal outcome parameters such as PIH, preeclampsia, and cesarean section were similar in both groups. The rate of elective labour induction was significantly higher in the control group than in the metformin group (72.7% vs. 38.9%, *P*=0.031). Elective labour induction was performed at 38 weeks of gestation in women on insulin therapy or with uncontrolled hyperglycaemia. However, this did not result in a higher rate of secondary cesarean section. There was no significant difference between the groups in the incidence of other pregnancy complications or adverse foetal or neonatal outcome.

Table 2 | Maternal and Neonatal Outcomes

Outcome	Metformin Group N=18	Control Group N=23	*P*-value*
Maternal Outcomes			
Gestation diabetes mellitus – no. (%)	10 (55.6)	16 (69.6)	0.355
Insulin therapy – no. (%)	2 (20)	8 (50)	0.218
Pregnancy induced Hypertension – no. (%)	2 (11,1)	0 (0)	0.187
Preeclampsia – no. (%)	0 (0)	2 (8.7)	0.495
Gestational age at start insulin – (wk)	24 ± 9.8	24 ± 5.3	0.185
Gestational weight gain[a] – (kg)	6.5 ± 4.7	6.8 ± 4.7	0.733
Labour induction	7 (38.9)	16 (72.7)	0.031*
Cesarean section	7 (38.9)	10 (45.5)	0.676
Primary	4 (40)	3 (42.9)	0.646
Secondary	6 (60)	4 (57.1)	0.646
Foetal or neonatal outcomes			
Intra-uterine foetal death[b] – no. (%)	0 (0)	0 (0)	
Early pregnancy loss[c] – no. (%)	0	1 (4.3)	0.561
Male sex – no. (%)	9 (50)	14 (63.6)	0.385
Gestational age at birth – (wk)	38.8	38.5	0.807
Birth weight – (gr)	3052 ± 441,4	3251.8 ± 486,6	0.539
Head circumference at birth – (cm)	33.6 ± 1.1	34.1 ± 2.4	0.300
Length at birth – (cm)	49.28 ± 2.2	49 ± 2.0	0.763
Large for gestational age[d] – no. (%)	1 (5,6)	0(0)	0.450
Pre-term birth (37 wk) – no. (%)	0 (0)	2 (8.7)	0.495
Apgar score < 7 at 5 min – no. (%)	1 (5.6)	2 (9.1)	0.577
Admission to the NICU – no. (%)	0 (0)	1 (4.8)	0.538
Birth trauma[e] – no. (%)	0 (0)	0(0)	
Hyperbilirubinemia requiring phototherapy – no. (%)	0 (0)	1 (4.8)	0.538
Respiratory distress syndrome – no. (%)	1 (5.6)	2 (9.5)	0.559
Hypoglycaemia – (< 2.6 mmol/L)	1 (5.6)	2 (9.1)	0.667

Data presented as mean ± SD or frequency (%); [a] Gestational weight gain was defined as the difference in weight between the fist antenatal visit (< 14 week) and the last antenatal visit; [b] Intra-uterine foetal death is any foetal death before onset of labor or no signs of life *in utero* after 20 weeks of gestation; [c] Early pregnancy loss: death < 20 weeks of gestation; [d] Large for gestational age was defined by a neonatal birth weight that was higher than the 90th percentile; [e]Birth trauma included shoulder dystocia and/or clavicle fracture and/or brachial plexus injury; *P*-value < 0.05 was statistically significant.

Adverse events

The incidence of Aes was higher in the metformin group but not significantly different (*P*=0.206) (Table 3). Most Aes were gastro-intestinal side effects related to the initiation of metformin. Two subjects stopped metformin (one due to hypoglycemia in combination with insulin and one due to gastro-intestinal effects). Two subjects temporarily stopped (maximum of 7 days) and one had doses

reduction to 500mg twice daily. The incidence of serious Aes (maternal and/or foetal) was higher in the control group (N=10 versus N=2) than in the metformin group but not significantly different (*P*=0.477).

Table 3 | Adverse Events

Category and event	Metformin Group N=18	Control Group N=23	P-value*
Serious adverse maternal events			0.477**
Hospitalization			
Abdominal trauma	1	0	
Abdominal pain without diagnosis?	1	0	
Threatening premature birth	0	1	
hyperemesis gravidarum	0	1	
hypovolemic shock due to placenta previa requiring cesarean section	0	1	
Preeclampsia requiring labour induction	0	1	
Partus immatures[a]	0	1	
Surgery			
Wrist trauma due to accident	0	1	
Herniation requiring laparoscopic surgery	0	1	
Serious foetal or neonatal adverse events			
Early foetal loss	0	1	
Congenital malformations			
type III laryngomalacy	0	1	
Neonatal infection requiring antibiotics	0	1	
Other maternal adverse events			
Gastro-intestinal effects	9	3	0.206
Gastro-intestinal effects resulting in doses reduction[b]	1	0	
Gastro-intestinal effects resulting in temporary treatment cessation[c]	2	0	
Gastro-intestinal effects resulting in metformin cessation[d]	1	0	
Hypoglycaemia in combination with insulin resulting in metformin cessation[e]	1	0	
Other events[f]	2	4	

[a] Early foetal loss at 18 weeks of gestation; [b] Doses reduced to 2d500mg from 20 weeks of gestation and continued until delivery; [c] One woman stopped metformin for 7 days and one woman for 3 days; [d] Stopped metformin at 20 weeks of gestation; [e] was used in combination with insulin, metformin stopped metformin at 35 weeks of gestation; [f] In the metformin group: headache (2x); control group: urinary tract infection (2x), pneumonia, asthma exacerbation; *P*-value < 0.05 was statistically significant; ** Serious adverse maternal and foetal/neonatal events were compared together.

Discussion

GDM is associated with significant maternal and perinatal morbidity. The prevalence of GDM is increasing in line with the obesity epidemic and causing considerable impact on health care services and economic burden. Therefore, the need for preventative strategies in women with high risk of GDM is vital. We hypothesized that metformin in women with high-risk factors, would reduce insulin resistance and would lead to a lower incidence of GDM. However, women in our study population were concerned about the effects of metformin use during pregnancy. Besides the general reluctance to take medication during pregnancy, the additional uncertainty about long-term safety had a negative impact on recruitment, and resulted in insufficient power to properly address outcome parameters. In this small study in women with a high risk of GDM, metformin had no significant influence on GDM incidence thus far. Our data did show a clear trend towards fewer women requiring additional insulin therapy in the metformin group than in the control group (20% vs. 50%). In the Metformin versus Insulin for the treatment of Gestational Diabetes (MiG) trial, the percentage of women with additional insulin therapy was higher (46.3%) than in our study population, despite the fact that the groups were similar in terms of BMI, treatment targets, and metformin dosages (17). Reducing the number of women on insulin therapy could potentially result in less maternal weight gain, fewer pregnancy complications, and less burden on specialist care (12). In our centre, insulin therapy is an indication for elective labour induction at 38 weeks of gestation, which resulted in significantly more women undergoing elective labour induction in the control group. However, this did not result in a higher rate of cesarean section as has been confirmed in previous studies (18-20). To date, two large randomized-controlled trials (The MOP trial and The EMPOWaR trial) studied the effect of metformin in obese pregnant women without diabetes mellitus (12,13). The EMPOWaR trial randomized white women with a BMI > 30, to metformin at a dose of 2.5 g per day or placebo, between 16–18 weeks of gestation. Results showed no difference in median birth weight, maternal weight gain, preeclampsia or other adverse neonatal outcomes between groups. Similar to our study, this study also had a low rate of eligible women who agreed to participate (13%). The lack of effect on birth weight could be attributed to low adherence to an adequate dose of metformin. In the MOP trial, women of all racial groups with a BMI > 35 were randomized to metformin 3.0 g per day or placebo between 12 to 18 weeks of gestation. Metformin was not associated with a lower incidence of median neonatal birth weight. However, women in the metformin group did have less gestational weight gain and a lower rate of preeclampsia compared with placebo, but the power was not adequate to study the effect on metformin on secondary outcomes. Women in the MOP trial had a higher adherence to an adequate of metformin than in the EMPOWaR trial (66% vs. 38%). Both studies concluded that metformin should not be given to obese pregnant women with diabetes. Perhaps improved high risk identification is necessary to identify a sub-group who will most likely benefit from an early intervention.

Metformin in pregnancy

Early reports on the use of metformin during pregnancy date back from the 1970s (21). Since then, sound evidence has shown the safety and efficacy of metformin use in pregnancy (6-9). A landmark study by Rowan et al. randomized 751 women with GDM to receive either metformin or insulin. They reported no significant differences in the composite foetal outcome between groups. Women treated with metformin had less weight gain compared to women treated with insulin (17).

Most data from metformin use in pregnancy has come from women with PCOS (11,22). One randomized controlled trial involving 273 women showed that metformin at 2.0 g per day or placebo in early pregnancy showed significantly less gestational weight gain than in the placebo group. However, there were no significant group differences in the rate of preeclampsia, GDM, preterm birth of birth weight (23). On the contrary, in a much smaller trial in women with PCOS, who received metformin at a dose of 1.7g per day, it was associated with a lower rate of preeclampsia (10).

There have been concerns about the effect of metformin on the male reproductive system. A study in rodents suggested that metformin treatment during pregnancy may have harmful effects on testicular development in offspring (24). However, recently Tertti et al. showed that prepubertal testicular size did not differ between offspring born to metformin-treated mothers and those born to insulin-treated mothers (25).

Overall, the short-term safety has been shown and there might even benefits but long-term safety data remains an issue. Follow-up data (MiG TOFU trial) in 2-year-old children with foetal exposure to metformin or insulin had similar birth weights but those exposed to metformin showed lower visceral fat than those exposed to insulin. This suggests a healthier fat distribution (26). In another follow-up study by Ro et al., eight year old children who were exposed to metformin *in utero*, showed no differences in weight, height, body composition, and insulin resistance. However, these children did have higher fasting glucose levels and higher systolic blood pressure (27). Despite lack of long-term safety going into adulthood, the National Institute for Health Care Excellence (NICE) guideline and the American College of Obstetricians and Gynecologists recommend the use of metformin in in GDM (28).

In conclusion, in confirmation with the available literature we observed that the use of metformin during pregnancy seems to have no harmful maternal or neonatal effects. We also observed in a small study population of in women with high risk for GDM that the use of metformin had no significant influence on GDM incidence. However, studies selecting those with the highest risk are necessary to reveille existing differences. In any case, longer-term follow-up of children exposed to metformin in pregnancy is essential to increase the acceptance of metformin as treatment but also meet recruitment of women with high risk in future clinical trials.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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PART III

POSTPARTUM AND BEYOND

CHAPTER 7

Investigating screening for diabetes in women with a history of Gestational Diabetes Mellitus

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is encountered more frequently in women with a history of gestational diabetes mellitus (GDM). Screening for T2DM after pregnancy is, therefore, recommended every ≥ 1–3 years in this population. Early detection could allow for timely intervention strategies, especially in women of childbearing age. Data on adherence to diabetes screening recommendations and the prevalence of T2DM in this population are not available in the Dutch population.

Aim: To investigate the T2DM screening rate and evaluate the risk of T2DM in the five-year period following the GDM pregnancy.

Methods: Single centre survey in 85 women diagnosed with GDM in 2010, using electronic medical records. Primary care physicians were asked to complete a survey regarding the screening frequency and the onset of T2DM within five years after GDM.

Results: On average 33% underwent yearly screening. The screening rate, however, went up to 61.2% after primary care physicians were requested to screen this population in 2015. Of the women who were screened 10 (19.2%) developed T2DM within 5 years after GDM.

Conclusion: Current screening recommendations are poorly met, leading to missed, or delayed diagnosis of T2DM in our population. T2DM is a frequent occurring long-term complication in those who were screened in the five year period after delivery. Optimizing awareness amongst health care professionals of GDM as a risk factor for T2DM is warranted, and strategies to improve surveillance are necessary.

Introduction

Type 2 diabetes mellitus (T2DM) is a major public health problem of epidemic proportion. The increasing incidence of T2DM is attributed to a global rise in obesity, growing elderly population and improved screening methods. In 2030 an estimated 66.5 million people will be diagnosed with diabetes in Europe alone (1). Identifying high-risk populations is essential for the initiation of screening and prevention in daily practice. Gestational diabetes mellitus (GDM) is associated with a seven-fold increased risk for T2DM when compared to non-diabetic pregnancies (2). Furthermore, their offspring are at increased risk for developing obesity and T2DM later in life (3,4). Without appropriate screening, T2DM often remains undiagnosed with asymptomatic progression. This is a concern, especially in young women of childbearing age, as undetected hyperglycaemia may cause early foetal loss or congenital malformations in a subsequent pregnancy (5). Evidence shows that intervention with lifestyle adjustments and diet are cost-effective and may prevent or delay the onset of T2DM (6,7). However, 6–12 weeks postpartum screening rates are low with only 20–45% of women with a GDM pregnancy return for screening (8,9). Little is known about adherence to long-term yearly screening recommendations. In the Netherlands, annual T2DM screening is recommended in the first five years after the GDM pregnancy (10). No data on adherence to these screening recommendations are available. The aim of this study was to investigate the T2DM screening rate and determine the long-term risk of T2DM in the five years following the GDM pregnancy.

Methods

We retrospectively analysed 85 women diagnosed with GDM in 2010 at the Maasstad Hospital, Rotterdam, the Netherlands. GDM was diagnosed when the fasting plasma glucose level was ≥ 7.0 mmol/L and/or 2-hour plasma glucose ≥ 7.8 mmol/L after the 75-g oral glucose tolerance test (11). Patient baseline characteristics were obtained from electronic medical records (Table 1). Body mass index (BMI) (kg/m^2) was determined in early pregnancy at the first visit to the obstetrics & gynaecology outpatient clinic. Insulin therapy was initiated in case dietary and lifestyle adjustments did not result in treatment targets (fasting glucose < 5.3 mmol/L and 2-hour postmeal < 6.7 mmol/L) (11).

In the Netherlands, primary care physicians are responsible for yearly T2DM screening in women with a history of GDM. According to the Dutch College of General Practitioners, yearly screening (fasting glucose) is advocated for the five-year period directly following the GDM pregnancy and every three years thereafter (10). In accordance with these recommendations we conducted a survey in 2015 among the primary care physicians of the 2010 cohort of 85 women with GDM. They were asked to verify two questions. 1) Did yearly T2DM screening (fasting glucose) during five years following the GDM pregnancy take place? 2) Was the woman diagnosed with T2DM in the five years following the GDM pregnancy? Diagnosis of T2DM was defined as: fasting glucose ≥ 7.0 mmol/L on two separate days, or fasting glucose ≥ 7.0 mmol/L or a random glucose ≥ 11.0 mmol/L in combination with symptoms associated with hyperglycaemia (10). If women had not been screened in 2015, then

the primary care physicians received a request to recommence T2DM screening. The percentage of T2DM in the five-year follow-up period was determined in those women who were screened every year (2010–2015) or in those who were only screened in 2015.

Results

In total 85 women diagnosed with GD in 2010 were analysed. Maternal characteristics are shown in Table 1. The median age was 33 with a range from 28-37. The majority of women (76.5%) had a BMI > 25 in early pregnancy. The population is diverse in terms of ethnicity: about one third is Caucasian, while 1 out of 4 women were of north-African descent. Insulin therapy was required in 45.9% of cases. Diabetes mellitus in a first degree family member (58.8%) appeared to be a prevalent risk factor in our population. Patient characteristics during pregnancy of the women who developed T2DM are shown in Table 2. In the first four years of follow-up, 33% of the population was screened yearly. In 2015, when the primary care physicians were requested to recommence screening, the rate increased to 61.2%. In the population of women who were screened in the five year follow-up period, 10 (19.2%) developed T2DM and 42 did not. In the group that was not screened (N=33), 11 women did not respond to screening invitation and 22 moved away, or changed primary care physicians or were lost to follow-up.

Table 1 | Patient characteristics

Parameter	No. (%)
Age (years)	
< 25	4 (4.7)
25–35	47 (55.3)
35–45	33 (38.8)
> 45	1 (1.2)
Age	33 [28–37]
BMI (kg/m^2)	7 missing
< 18,4	1 (1.2)
18,5–24	12 (15.4)
25–29	34 (43.6)
30–34	14 (17.9)
≥ 35	17 (21.8)
BMI	28.4 [26–33]
Race/ethnicity	6 missing
Caucasian	25 (29.4)
Negroid	11 (12.9)
Asian	2 (2.4)
Hindu	6 (7.1)
North-African	20 (23.5)
Turkish	9 (10.6)
Middle East	2 (2.4)
Other	4 (4.7)
First degree family member with DM	50 (58.8)
First trimester random glucose (mmol/L)	5.2 [4.6–5.6]
75-g OGTT: fasting glucose – mmol/L	5.7 ± 1.7
75-g OGTT: 2-hour glucose – mmol/L	8.7 ± 1.5
Gestational age at GD diagnosis (weeks)	28 [23–32]
Insulin therapy	39 (45.9)

BMI: body mass index; DM: Diabetes Mellitus; OGTT: oral glucose tolerance test; GDM: gestational diabetes mellitus
Data are presented as frequency (%), median [IQR] or mean ± SD.

Table 2 | Patient characteristics during pregnancy of women who developed T2DM

Parameter	T2DM (N=10)	No T2DM (N=42)
Age (years)	33 [31–40]	33 [29–38]
BMI (kg/m^2)	32.1 [27–36]	28.5 [26-32]
Race/Ethnicity		
Caucasian	3 (10)	17 (40.5)
Negroid	1 (10)	6 (14.3)
Asian	1 (10)	1 (2.4)
Hindu	1 (10)	2 (4.8)
North-African	2 (20)	9 (21.4)
Turkish	1 (10)	4 (9.5)
Middle East	1 (10)	2 (4.7)
Other	0 (0)	1 (2.4)
First trimester random glucose (mmol/L)	5.05 ± 0.75	5.5 ± 1.2

BMI: Body Mass Index; Data are presented as frequency (%), median [IQR], or mean ± SD.

Discussion

To our knowledge this is the first study, investigating adherence to long-term diabetes screening recommendations in women with a history of GDM in the Netherlands. Our study shows that the mandatory screening of women with GDM on the development of overt T2DM is suboptimal at best. Furthermore, T2DM was a frequent complication in those who were screened. Similar low screening rates have been reported before (12). Sending a reminder to the primary care physicians seems to have a significant effect on the screening rate, suggesting that reminders could help to accomplish higher screening rates. There are a number of explanations as to why long-term screening recommendations are not met. Post-partum screening studies have shown that women fail to return for screening (20–45% attendance) shortly after pregnancy (8,9). Reported barriers for postpartum screening included: limited time and other priorities such as childcare (13,14). If women with GDM do not attend post-partum screening programs, then they often remain out of 'screening' sight in the subsequent years after pregnancy. There are several explanations for the low long-term follow-up testing rates. Firstly, the lack of adequate transition of care from the endocrinologist and/or obstetrician during pregnancy to the primary care setting after delivery remains a pitfall in accomplishing proper screening. Secondly, conflicting screening programs on long-term follow-up frequency exist internationally, promoting ambivalence towards systematic screening (15,16). The American Diabetes Association recommends diabetes screening 6–12 weeks postpartum using OGTT and every 1–3 years thereafter (15). Women should be screened every 3 years if the results are normal; however, if impaired glucose tolerance or impaired fasting glucose is detected, screening should be done annually. The American College of Obstetrics and Gynaecology recommends

screening 6 weeks postpartum, but does not provide recommendations after this period (16). The Dutch Obstetrics and Gynaecology Association recommends screening 6 weeks postpartum and yearly screening thereafter, but no statement is made about the duration of follow-up (11).

Finally, recognition and awareness of GDM as a risk factor for diabetes is not widespread among patients and health care providers. In a long-term follow-up study more than half of the women with a history of GDM reported that they had not been informed about their risk of T2DM (17). In a survey among women 3–5 years after their GDM pregnancy, less than half believed that it was "highly possible" or "very possible" that they would develop T2DM (18). Another survey showed that although almost all women with a history of GDM were aware of the risk for diabetes, only 16% believed that they were at risk as an individual (19).

Improving screening rates is important for a number of reasons. First, early detection could allow for timely intervention. Lifestyle intervention and metformin reduced progression to T2DM compared with placebo by 35% and 40%, respectively (6). Additionally, considering the childbearing age of this population, it is important to aim for glycaemic control before future pregnancies.

Since the number of screened women was relatively small, the risk of T2DM in our population should be interpreted with caution. Although T2DM is a frequent long-term complication in women with GDM, the percentage of T2DM in our population appeared to be lower than previously reported (20). However, our estimates are in line with more recent data from a systematic review showing a risk between 9.5% and 37% (3.5–11.5 years follow-up) (21). In our study, the risk of T2DM was determined in those women with a complete five-year follow-up or with T2DM screening in 2015. Theoretically, women with pre-gestational diabetes could have been included, however, since the first trimester screening showed normal random glucose levels this is less likely. The need for insulin therapy was higher in our population than previously described (22). This could be attributed to strict multidisciplinary management policy. Insulin therapy was initiated if glycaemic targets were not met in two consecutive days. Furthermore, obesity was highly prevalent in our population (23). Other limitations of this study include the retrospective design, and prepregnancy BMI was not available.

Potential strategies to improve surveillance

As education regarding the risk of T2DM during pregnancy will probably result in improved awareness and self-management after pregnancy, counselling women about long-term screening and raising awareness is clearly needed. Correspondence with clear screening recommendations from the gynaecologist or endocrinologist to the primary care physician is a vital step in the transfer of care. Subsequent registration of the GDM diagnosis in primary care medical record systems is important for the identification of women who should be screened for T2DM after pregnancy. If GDM is not registered in the international disease codes (International Classification of Primary Care – ICPC), or a high-risk label is given, then automatic yearly screening invites will not be generated and annual screening will likely not be performed. This is particularly worrisome in those patients who do not initiate screening themselves. Through registration in electronic medical records, screening could be implemented on a large scale by means of automated yearly reminders. Furthermore, uniformity in international long-term screening guidelines should be met for the implementation

of systematic screening. Several types of reminding systems have been investigated. Women prefer SMS reminding according to a questionnaire in Australian cohort. Postal and voice calls were the least preferred types (14). A systematic review investigated the effect of reminder systems for postpartum screening. Results showed that direct telephone calls strengthened the reminding effect on the women. Surprisingly, reminding both the primary care physician as well as the patient has not proven to be effective (24). A recent study showed that introducing a regional central coordinator to remind women both in writing and verbally improved post-partum screening rates to 75% (25). Furthermore, identifying those women who are at greatest risk of developing diabetes postpartum would allow for better individual education during pregnancy. Maternal age, obesity, insulin therapy, highest fasting glucose level (4^{th} quartile vs. 1^{st} quartile range), severity of glucose intolerance and a previous GDM pregnancy have been reported to be predictive factors for the development of T2DM (26,27).

In summary, current screening recommendations appear to be largely unsuccessful, leading to missed diagnoses of T2DM in women of childbearing age. T2DM is a frequent long-term complication in those women who were screened. Optimizing awareness amongst health care professionals of GDM as a risk factor for T2DM is warranted and strategies such as systematic reminder systems are necessary to improve surveillance.

Disclosures

All authors have no conflicts of interest.

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CHAPTER 8
DISCUSSION

Discussion & future perspectives

This thesis tries to address the overall management of gestational diabetes mellitus (GDM). We investigated the role of biomarkers and risk stratification, evaluated current management, and performed long-term follow-up in women with a history of GDM. This chapter discusses the main findings of this thesis, explores clinical implications, and provides recommendations for future research.

Biomarkers and risk stratification

The overall incidence of GDM is increasing worldwide in part due to the increasing incidence of obesity and advanced maternal age (1). Epidemiological, clinical and experimental studies have shown that adverse exposures during foetal development, (over- and under nutrition) are associated with increased susceptibilities for many adult diseases (2-4). This creates a vicious cycle in which children exposed to hyperglycaemia in utero may be more likely to develop obesity and metabolic diseases later in life, and pass on the adverse environmental exposure to the next generation (5,6). Therefore, pregnancy offers a window of opportunity to not only reduce or prevent GDM and related complications but it also has potentials to prevent intergenerational transfer of adult diseases such as diabetes and cardiovascular disease. The increasing prevalence, long-term implications and rising overall health care costs, make GDM a public health concern (7).

While current screening practices are largely based on the presence of maternal risk factors, in practice they fail to adequately identify all women with GDM (8,9). Moreover, recent prospective data has shown that diagnosis of GDM is preceded by foetal overgrowth occurring between 20–28 weeks' gestation, and that this is exacerbated by maternal obesity. This provides rationale for early pregnancy risk stratification and preventative strategies to ultimately prevent GDM and adverse outcome (10).

GDM is increasingly being recognised as an inflammatory condition (Chapter 2). Cytokine production by macrophages in the adipose tissue affects post receptor insulin signalling. This disturbance of insulin signalling results in increased insulin resistance (11). Prospective studies have shown that a down-regulation of anti-inflammatory cytokines (i.e. adiponectin) and up-regulation of pro-inflammatory cytokines (i.e. tumour necrosis factor- alpha, interleukin -6) are linked to GDM (12,13). Adiponectin appears to be one of the most promising candidates in the prediction of GDM (14). The addition of biomarkers to current screening models, which precede hyperglycaemia, might improve predictive value. Data from the largest study to date, investigating biomarkers and clinical risk prediction tools, showed that previous GDM, age, systolic blood pressure, sum of maternal skinfold thicknesses and anthropometric ratios (waist:height and neck:thigh) showed good discrimination (AUC 0.71, 95% CI 0.68–0.74). This improved with addition of candidate biomarkers to 0.77 (95% CI 0.73–0.80) ($P < 0.001$). Candidate biomarkers contributing to this model were HbA1c, glucose, fructosamine, triglycerides, adiponectin and sex hormone binding globulin (15).

Overall, evidence thus far remains contradictory due to difference in study designs, assay methods, diagnostic criteria for GDM, and certain confounders. The clinical implementation of clinical prediction models with biomarkers will depend on the additional predictive value, cost-effectiveness and reduction of pregnancy complications. Prospective studies are warranted to confirm that biomarkers are independently associated with GDM risk. We are currently undertaking a study entitled "Adipokines in the Prediction of GDM". The first aim of this study is to examine the association of adiponectin levels and the risk of developing GDM. Secondly, if adiponectin is associated with GDM, then we aim to investigate the additional predictive ability of adiponectin in a clinical risk prediction tool (ethnicity, family history, history of GDM and body mass index) based on a previous study by van Leeuwen et al. (16).

In the process of further unravelling the pathophysiology of GDM, biomarkers associated with insulin resistance are to be explored. To date, the value of acylated ghrelin (AG) and unacylated ghrelin (UAG) as biomarkers in GDM was unknown. Ghrelin is a stomach-derived peptide discovered in 1999 as the endogenous ligand for growth hormone secretagogue-receptor (GHS-R) (17). Ghrelin is involved in a wide range of biological functions including glucose homeostasis (18). We investigated the potential role of ghrelin as a biomarker in GDM, using a double-antibody sandwich ELISA (Chapter 3). We hypothesized that ghrelin levels would be lower in women with GDM as a result of hyperinsulinaemia and increased insulin resistance. Contrary to our hypothesis, we demonstrated that ghrelin levels did not differ between women with GDM and normal glucose tolerance. Ghrelin levels decreased post oral glucose load showing a physiological response. Other reports have yielded conflicting results, with some presenting decreased ghrelin levels while others showed no effect of GDM on ghrelin levels (19-23). However, most studies measured total ghrelin, without differentiating between AG and UAG (24). We concluded that ghrelin is not a useful biomarker in GDM.

Evaluating current management of gdm

Maternal and Neonatal Outcomes

The national guideline "Diabetes and Pregnancy" for screening and treatment of GDM was introduced by the Dutch Society of Obstetrics and Gynaecology in 2010 (25). In the period 2010 to 2015, we observed a five-fold increase in the number of women treated for GDM at the Maasstad Hospital diabetes outpatient clinic. This is most likely due to the implementation of the guideline and ongoing sociodemographic changes. In light of this, we performed an analysis of pregnancy outcomes in women treated for GDM between 2010 and 2015 in a large multi-ethnic cohort in Rotterdam (Chapter 4). The aim was to evaluate maternal and neonatal outcomes and identify factors associated with neonatal complications. We showed that the current national screening and treatment guideline achieved a well-controlled GDM population with low rate of adverse pregnancy outcomes. One could argue that we assessed treatment outcomes in a more 'T2DM' population rather than a GDM population based on the relatively 'outdated' WHO 1999 diagnostic criteria (75-g OGTT fasting glucose ≥ 7.0 mmol/L; 2-h glucose level ≥ 7.8 mmol/L) (26). In this population with higher levels of hyperglycaemia one might expect higher rates of adverse pregnancy outcomes.

However, treatment targets (fasting < 5.3 mmol/L, 2-h postprandial glucose level < 6.7 mmol/L) were according to current international guidelines which might explain low incidence of adverse outcomes (25).

Obesity and insulin therapy were independently predictive of neonatal complications. Our GDM pregnancy outcomes are in line with the results of several systematic reviews showing that treatment of GDM results in lower rates of adverse outcomes (27-29). The rate of large-for-gestational-age (LGA) (8.5%) infants was low but similar to other landmark treatment trials (30,31). Overall differences in pregnancy outcomes can be attributed to different GDM diagnostic criteria and definition of LGA used in other studies. Moreover, weekly monitoring and strict initiation of insulin therapy might also contribute to a low rate of adverse outcomes in our study. In contrast, Koning et al. reported a higher proportion of LGA infants (19.9%) despite using the same LGA definition (adjusted for age, parity and ethnicity) and treatment guideline (32). Firstly, women with GDM in their cohort were slightly more obese (38.2% vs. 33.9%) compared with our GDM population. Secondly, authors used a 1-hour postprandial glucose target (< 7.8 mmol/L) instead of a 2-hour postprandial glucose target (< 6.7mmol/L). Thirdly, although the overall labour induction rate (38 + 0 and 39 + 0 weeks of gestation) was higher in their cohort; they did not perform labour induction in all women with insulin therapy.

The rate of labour induction was also higher in our population compared to the general obstetric population. In our cohort women who were treated with insulin therapy underwent elective labour induction between 38 + 0 and 39 + 0 weeks of gestation. Current delivery management is based (elective induction compared with expectant management) on one randomized-controlled trial (33). Results from the GINEXMAL trial will provide additional evidence as to whether or not, in women with GDM, induction of labour between 38 + 0 and 39 + 0 weeks is an effective management to improve maternal and neonatal outcomes (34).

Adoption of the IADPSG diagnostic criteria: is it time?

The current diagnostic criteria for GDM are based on the World Health Organization (WHO) 1999 criteria (75-g OGTT: fasting glucose ≥ 7.0 mmol/L; 2-h postprandial glucose ≥ 7.8 mmol/L) (26). Our results indicate that the rate of adverse pregnancy outcomes under the current national screening and treatment guideline is low. This is an important finding given the current discussion regarding the adoption of the more stringent diagnostic criteria, as proposed by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) (75-g OGTT: fasting glucose ≥ 5.1 mmol/L; 1-h glucose level ≥10.0 mmol/L; and 2-h postprandial glucose ≥ 8.5 mmol/L) (35). These criteria have been endorsed by global health organizations in recent years (36,37).

Critics opposing the IADPSG criteria suggest that these strict criteria lead to ´over-estimation' of the prevalence (~20%), resulting in unnecessary medicalisation of pregnancy. Furthermore, they state that evidence for the association between these glycaemic levels and complications is weak (38,39). Arguments in favour suggest that considering the adverse metabolic alterations in utero caused by impaired glucose metabolism, more stringent criteria are necessary (2). Furthermore, other criteria failed to address the link between the worldwide increasing prevalence of glucose intolerance, T2DM and GDM. The National Health and Nutrition Survey (NHANES) in 2005–2008, showed that

30% of women in childbearing age in the U.S. have impaired glucose metabolism, suggesting that de IADPSG prevalence estimate is not so high after all (40). Careful consideration must go into adapting current guidelines, as the adoption could overwhelm health care services and increase economic burden (41). The IADPSG criteria will identify a GDM population with mild hyperglycaemia. However, we cannot automatically translate the proven treatment benefits to this new population (42,43). Currently, there are no evidence-based principles regarding the management of these additional women with GDM. Do the same management guidelines apply? Would dietary and lifestyle counselling be sufficient? Furthermore, the treatment of women with 'mild' hyperglycaemia may increase the risk of maternal hypoglycaemia and foetal undernourishment, which in turn might increase the risk of metabolic disorders in adult life (4,44). Controversy regarding the screening and management of GDM will remain until sound evidence from intervention studies is provided.
To date, retrospective analysis and observational studies have shown potential benefits of treating mild hyperglycaemia (45). Future recommendations include large well-designed prospective trials including cost-effectiveness before guidelines are revised (46). Furthermore, the IADPSG criteria would only be cost-effective if linked to aggressive postpartum follow-up with intense diet and lifestyle management to reduce future risk of type 2 diabetes mellitus (T2DM) (41).

Obesity

More than a third of our GDM population was obese in the first trimester of pregnancy. Obesity was the second most prevalent risk factor for GDM and independently associated with the neonatal complications. Meta-analysis showed an unadjusted OR for developing GDM of 2.14 (95% CI 1.82–5.53), 3.56 (3.05–4.21), and 8.56 (5.07–16.04) in overweight, obese and severely obese women respectively compared to normal weight pregnant women (47). Obesity is associated with increased risk of significant adverse maternal and neonatal outcomes (48). Data from the HAPO study showed that the rate of macrosomia (birth weight > 4000 g) was twice as high in GDM compared to non-GDM in women with and without obesity. Obesity and GDM are therefore considered independent predictors of macrosomia. Furthermore, children born from obese mothers show higher rates of admission to the neonatal department tend to be large for gestational age and their lifetime risk of obesity and T2DM is increased (49-51). Prevention and strategies to reduce obesity, especially in women of childbearing age, are needed to overcome this vicious cycle. Interventions in pregnant obese women have shown little effect on GDM and the incidence of LGA infants (52-54). Perhaps risk stratification in obese women is necessary to identify a group that will most likely benefit from intervention. Furthermore, we suggest that women of childbearing age are offered evidence-based and personalised lifestyle- and nutrition intervention before the pregnancy period (55,56). For example, a program such as the mobile health coaching program www.SmarterPregnancy.co.uk (Dutch version www.SlimmerZwanger.nl) is presented as an opportunity to promote preconception health (57). This should be implemented to support healthcare professionals in delivering 'nutrition and lifestyle care' in routine patient care (58). Further awareness about preconception health should be promoted through government programs and campaigns.

Monitoring of treatment

Monitoring the effect of treatment during pregnancy is essential for pregnancy outcomes to be improved. Current practice consists of self-monitoring of blood glucose by means of finger-stick blood glucose measurements (59). We evaluated current monitoring of treatment in women with GDM (Chapter 5). In this study, we showed that blinded continuous glucose monitoring (CGM) detected hyperglycaemia in women with a normal self-monitoring of blood glucose profile. The percentage of time in hyperglycaemia detected by blinded CGM was however, not circumstantial. Due to study design and sample size, the clinical value of CGM versus SMBG, for improving pregnancy outcome could not be assessed. Recent evidence from the CONCEPTT trial showed that CGM during pregnancy in patients with type 1 diabetes was associated with improved neonatal outcomes (60). We suggest that in order to advance treatment monitoring research, uniformity in CGM parameter analysis should be reached (61). Results from the GlucoMoms trial might give more insight into the role of blinded CGM in women with insulin-treated GDM. However, investigators used intermittent blinded-CGM for brief periods during pregnancy, which raises the question of whether the effect on pregnancy outcome will be adequately studied (62). Future study recommendations should also focus on the evaluation of technological innovations. Technological innovations such as telemedicine (health services at a distance using a range of technologies) and personalized and interactive apps/mobile health programs present opportunities to improve accuracy, efficiency, patient satisfaction and economic benefit (63). At this point there is insufficient evidence to pose that telemedicine might improve pregnancy outcome in women with GDM but no harmful effects have been described either (64). Upcoming results from RCTs will most likely provide the first robust answer of the potential for integrated remote monitoring system in women with GDM (65,66).

Pharmacological prevention of GDM

The prevention of hyperglycaemia during pregnancy can have several positive effects: a reduction in short-term pregnancy complications, a reduced risk of long-term sequelae for mother and child, and a decrease in the economic burden (67). In view of the striking increase in obesity, women with a high risk of GDM become the ideal group for targeting primary prevention strategies. To date, attempts at reducing the incidence of GDM have focused on dietary and lifestyle intervention, but have been largely unsuccessful (52,54,68-70).

Metformin, a biguanide analogue, decreases hepatic glucose production and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Most data of the effect of metformin on pregnancy outcome has come from women with polycystic ovary syndrome (PCOS) (71,72). Data has shown conflicting evidence for the effect of metformin on the incidence of GDM and pregnancy outcomes (72-76).

We investigated the effect of metformin in women with a high risk of GDM (Chapter 6). High risk was defined according to the screening criteria provided by the national guideline (25). Our hypothesis was that metformin in early pregnancy (started at 14 weeks of gestation) would reduce the progressive insulin resistance and subsequent onset of GDM. In this small study, the rate of GDM in both groups was not significantly different but groups were too small to draw sound conclusions. However, we observed no harmful effects of metformin use in pregnancy on either mother or child.

Two outcomes were of particular interest. Firstly, the rate of elective labour induction was significantly higher in the control group than in the metformin group. Elective labour induction was performed at 38 weeks of gestation in women requiring insulin therapy or with uncontrolled hyperglycaemia. However, this did not result in a higher rate of secondary caesarean section or other adverse neonatal outcomes. Secondly, although the study groups were small, our data did show a clear trend towards fewer women requiring additional insulin therapy in the metformin group than in the control group (20% vs. 50%). Besides the reluctance among pregnant women to take medication in general, the additional uncertainty about long-term safety of metformin use on offspring had a negative impact on recruitment. A study examining the use of metformin throughout pregnancy in 109 women with PCOS found normal growth and motor development in infants at 18 months old (77). The MiG-TOFU trial evaluated body composition in 2-year old children who were exposed to metformin and/or insulin during pregnancy. Authors reported a more preferable body fat distribution in children exposed to metformin, but total or body fat percentage was the same in both groups (78). Follow-up studies evaluating in the effect of metformin into adolescence and adulthood are urgently needed.
Two recent RCTs studying the effect of metformin in specifically non-diabetic obese pregnant women concluded that, metformin had no effect on the incidence of GDM and birth weight (53, 79). In the MOP trial, metformin was associated with less gestational weight gain and a lower rate of preeclampsia (79). The lack of effect on birth weight, despite lowering maternal glucose levels might raise the question whether other factors besides maternal hyperglycaemia drive neonatal adiposity. Catalano et al. suggests that excess maternal lipids might play an important role in foetal adiposity, particularly in the presence of maternal obesity (80). Perhaps inflammatory markers are facilitating foetal fat accumulation? Other mechanisms besides maternal hyperglycaemia, resulting in foetal adiposity and metabolic dysfunction should be investigated.
Overall, further research is warranted to identify the effective means of lifestyle modification and/or pharmacologic intervention, both in the pre-conception period and during pregnancy. Moreover, prediction tools to identify a sub-group within the high-risk population whom are most likely to benefit from early intervention are needed.

Postpartum and beyond

Type 2 diabetes mellitus screening

Undetected hyperglycaemia postpartum could also lead to an increased risk of congenital malformations or early pregnancy loss in a subsequent pregnancy (81). Furthermore, hyperglycaemia during early stages of foetal development may cause a predisposition to long-term pathology in offspring creating a vicious cycle (2,82). Glucose intolerance resolves after pregnancy in most women, but the risk of developing T2DM is estimated at 50%–70% in the 5–10 years after delivery (83).

Lifestyle interventions may prevent or delay the onset of T2DM and provide opportunity for early initiation of treatment and prevention of complications (84,85). Therefore, postpartum and long-term T2DM screening is advised in this population.

In clinical practice, the loss of women with GDM to follow-up screening after delivery is a significant problem (86). According to the Dutch College of General Practitioners, yearly diabetes screening is recommended in the first five years after a GDM pregnancy (87). In our follow-up study we showed that only about a third of the population was screened annually during the first five years after pregnancy (Chapter 7). However, sending a reminder to the general practitioners had significant impact on the screening rate. Of the women who were screened annually, about 20% developed T2DM in the five years after GDM. Similar low screening rates were reported by Koning et al. (88). Our results indicate that awareness about GDM as a risk factor for T2DM is low amongst both women and health care providers. Suggestions to improve diabetes screening rates include: correspondence with clear screening recommendations from the endocrinologist to the general practitioner and implementing track systems with automated yearly screening invites. Furthermore, women should be counselled about their risk of diabetes during pregnancy and be advised to also take own responsibility for screening.

Identification of those with the highest risk might allow for better risk-stratification in daily practice. Various risk factors for postpartum diabetes, including age, glucose level in pregnancy, insulin treatment, family history of diabetes and obesity were identified in previous studies (89). Furthermore, there is mounting evidence that breastfeeding has short- and long-term health benefits for mothers with GDM. Results of observational studies and a small number of prospective studies suggest that breastfeeding is associated with improvements in glucose and lipid metabolism together with reduced risk of T2DM in women with GDM (90). Training, education, and support should be provided to promote breastfeeding among women with GDM.

The Diabetes Prevention Program outcomes study (10-year follow-up) showed that in women with a history of GDM, intense lifestyle intervention and metformin reduced progression to diabetes compared with placebo by 35% and 40%, respectively (84). A strong determinant of the success was the availability of personal exercise trainers, but these require considerable financial resources. Maternal BMI and pregnancy-induced hypertension are also associated with on the future health of the women and their offspring. Postpartum lifestyle interventions focussed not only at glucose intolerance, but also weight and blood pressure appears necessary (91).

In conclusion, surveillance strategies should be improved and women with postpartum glucose intolerance should be offered an intervention.

Future research recommendations include performing studies that prospectively compare different strategies to improve patient and doctor adherence with screening recommendations. Specifically, investigating what the most effective health information technology intervention is.

Conclusion

In conclusion, in this thesis we have shown that ghrelin is not a useful biomarker in gestational diabetes mellitus. The treatment of GDM according to the current national guideline is successful in achieving a low rate of adverse pregnancy and perinatal outcome. Blinded continuous glucose monitoring showed limited additional diagnostic value compared with self-monitoring of blood glucose in women with well-controlled GDM. Metformin in women with a high risk of GDM showed no effect on GDM incidence but larger studies are needed, no harmful effects have been observed thus far. We have also demonstrated that current long-term type 2 diabetes screening in women with GDM is suboptimal and that improved surveillance strategies are necessary.

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Part IV

Summary and Appendices

CHAPTER 9

SUMMARY

Gestational diabetes mellitus (GDM) is one of the most common metabolic disorders during pregnancy. The number of pregnancies complicated by GDM is increasing in line with the obesity epidemic. Advanced maternal age, certain ethnicities (e.g. Asian, Caribbean), family history of diabetes mellitus, GDM in a previous pregnancy and obesity are among others risk factors for developing GDM. Untreated GDM leads to increased risk of maternal and perinatal complications such as preeclampsia, caesarean section, pre-term birth, excessive birth weight (> 4000 gram), neonatal hypoglycaemia, and neonatal jaundice. Moreover, women with a history of GDM are at increased risk of developing type 2 diabetes mellitus and cardiovascular disease after pregnancy. Children born from mothers with GDM have higher chances of developing obesity and type 2 diabetes mellitus. In general, GDM is diagnosed between 24–28 weeks of pregnancy by means of a 75-g oral glucose tolerance test. Treatment consists of dietary and lifestyle advice. If these measures fail to achieve glycaemic goals, insulin is initiated. The importance of treating women with GDM is now widely accepted and has led to a reduction in serious pregnancy complications. Consensus regarding screening methods is still lacking.

GDM is an emerging area of research and many questions regarding pathophysiology, prevention, management and long-term follow-up remain unanswered.

Firstly, understanding the pathophysiology of GDM is essential for identifying mothers at risk, implementing preventative strategies and providing optimal management. However, in current antenatal care, understanding of pathophysiology of GDM is limited. Current risk prediction is mostly based on maternal history and clinical risk factors and may not optimally identify high risk pregnancies. Exploring the pool of biomarkers could advance understanding of GDM pathophysiology and explore risk prediction. Secondly, there is ongoing (inter)national debate regarding the adoption of the more stringent International Association of Diabetes and Pregnancy Study Groups (IADPSG) diagnostic criteria, which have been endorsed by several international organisations, but have not been implemented in the Netherlands. In light of this, we investigated pregnancy outcomes in women screened and treated according to the current Dutch Society of Obstetrics and Gynaecology guideline "Diabetes and Pregnancy" which was implemented in 2010. We also aimed to investigate whether the current treatment monitoring technique by means of self-monitoring of blood glucose was sufficient for the detection of hyperglycaemia in women treated for GDM. Thirdly, while we have put extensive effort into managing glucose levels during pregnancy, less effort and success has been realized in achieving postpartum and long-term diabetes screening. The national guideline recommends diabetes screening postpartum and annually in the five years following the GDM pregnancy. Data on adherence to these screening recommendations was not available for the Dutch population.

This thesis aimed to investigate the overall management of GDM including: biomarkers and risk stratification, evaluating current management, and long-term follow-up of women with GDM.

In part I of this thesis we will explore the literature on GDM and biomarkers, to enhance understanding of pathophysiology and explore risk prediction, with a goal to ultimately promote prevention of GDM.

In **Chapter 1** we provide the background and introduction of this thesis.

In **Chapter 2** we give an overview of the literature regarding the potential role of biomarkers in the prediction of GDM. Low-grade inflammation and insulin resistance are exacerbated in women with GDM. Studies have shown that GDM is linked to the down-regulation of adiponectin and anti-inflammatory cytokines (e.g., IL-10) and up-regulation of leptin and pro-inflammatory cytokines (e.g., IL-6 and TNF-α) implicated in insulin resistance. Integrated assessment in early pregnancy with a prediction model based on biomarkers & clinical parameters may enhance identification of high risk pregnancies. We are currently investigating the association between adiponectin and GDM and aim to evaluate the addition of adiponectin in a clinical risk prediction rule for GDM.

In **Chapter 3** we studied ghrelin levels in pregnant women with GDM and normal glucose tolerance, using a double anti-body sandwich ELISA kit. Ghrelin plays an important physiological role in modulating growth hormone secretion, insulin secretion and glucose metabolism. Our hypothesis was that women with GDM have more insulin resistance and hyperinsulinaemia with subsequently lower ghrelin levels than women with normal glucose tolerance. Contrary to our hypothesis, ghrelin levels did not significantly differ between women with GDM and normal glucose tolerance. Furthermore, ghrelin levels decreased significantly after oral glucose ingestion, showing a physiological response. These results indicate that ghrelin levels are not affected by GDM. This implicates that ghrelin levels are not useful as a predictive biomarker of GDM.

In **part II** of this thesis we evaluated current management of women with GDM.

In **chapter 4** we studied maternal and neonatal outcomes in a large multi-ethnic cohort in Rotterdam. We compared this GDM cohort with a general obstetric population (no pregestational diabetes or GDM) obtained from the National Perinatal Registry. We showed that BMI ≥ 30, OGTT levels (fasting and 2-h), GDM in a previous pregnancy and (Mediterranean) ethnicity were independent predictors for the need of insulin therapy. We also showed that obesity and insulin therapy are independently predictive of an increased risk of neonatal complications. The rate of labour induction was relatively high without leading to increased rates of caesarean section. Perhaps this contributed to the striking low rate of large-for-gestational-age infants. Overall, we concluded that the current national screening and treatment guideline achieved a well-controlled GDM population with low rate of adverse pregnancy outcomes.

The goal in treating GDM is to reach normal glucose levels and reduce the risk of related adverse pregnancy outcomes. Current monitoring of treatment is performed by self-monitoring of blood glucose (SMBG). In **chapter 5** we evaluated the potential of blinded continuous glucose monitoring (CGM) for the detection of high glucose levels (hyperglycaemia) in women treated for GDM. We observed that blinded CGM improved the detection of hyperglycaemia slightly compared with SMBG but the duration was not circumstantial.

In **chapter 6** we investigated the effect of metformin in women with a high risk of GDM. Our hypothesis was that metformin in early pregnancy would reduce insulin resistance and subsequently the incidence of GDM. Due to recruitment problems we were not able to study the primary outcome but we observed no harmful effects of in women and offspring. Long-term follow-up in children exposed to metformin in pregnancy is necessary for widespread acceptance by clinician's and patients.

In **part III** we evaluated long-term diabetes screening recommendations.

In **Chapter 7** we studied long-term follow-up of women with GDM in the primary care setting. The aim was to evaluate diabetes screening in the five years following the GDM pregnancy. We demonstrated that only about a third of the women are screened annually after pregnancy. After sending a reminder to the general practitioners, the screening rate nearly doubled that year. Optimizing patient and healthcare provider awareness is necessary and improving surveillance strategies is warranted.

In the general discussion we interpret the findings of the studies described and their implications for clinical practice **Chapter 8**. We also provide perspectives for future research.

Conclusion

In this thesis we have shown that ghrelin is not a useful biomarker in gestational diabetes mellitus (GDM). The treatment of GDM according to the current national guideline is successful in achieving a low rate of adverse pregnancy and perinatal outcome. Blinded continuous glucose monitoring showed limited additional diagnostic value compared with self-monitoring of blood glucose (SMBG) in women with well-controlled GDM. Metformin in women with a high risk of GDM showed no effect on GDM incidence but larger studies are needed, no harmful effects have been observed thus far. We have also demonstrated that current long-term type 2 diabetes screening in women with GDM is suboptimal and that improved surveillance strategies are necessary.

CHAPTER 10
SAMENVATTING

Zwangerschapsdiabetes wordt definieert als verhoogde bloedglucosewaarden die voor het eerst tijdens de zwangerschap worden ontdekt. Zwangerschapsdiabetes komt ongeveer in 1–14% van alle zwangerschappen voor. De laatste decennia neemt wereldwijd de prevalentie van overgewicht en obesitas sterk toe, dit levert mede een belangrijke stijging op van het aantal zwangeren met zwangerschapsdiabetes. Onbehandelde zwangerschapsdiabetes leidt tot een verhoogde kans op het ontwikkelen van complicaties tijdens de zwangerschap. Complicaties kunnen optreden bij zowel moeder als kind en zijn o.a. zwangerschapsvergiftiging, een keizersnede, een kind met een hoog geboortegewicht (> 4000 gram), een te laag bloedglucose en geelzucht bij het pasgeboren kind. In de jaren na de zwangerschap hebben vrouwen een verhoogd risico op het ontwikkelen van diabetes mellitus type 2 en hart en vaatziekten. Tevens hebben de kinderen van moeders met zwangerschapsdiabetes een verhoogd risico op het ontwikkelen van obesitas en type 2 diabetes mellitus op latere leeftijd. Risicofactoren voor het ontwikkelen van zwangerschapsdiabetes zijn o.a: hogere leeftijd van de moeder, niet-westerse afkomst, diabetes mellitus type 2 in de familie, zwangerschapsdiabetes in een eerdere zwangerschap, en obesitas. Bij vrouwen met een verhoogd risico op zwangerschapsdiabetes wordt meestal gescreend tussen de 24ste en 28ste week van de zwangerschap door middel van een 75-g orale glucose tolerantie test. Er is tot op heden geen internationale consensus betreft de screening en diagnostiek van zwangerschapsdiabetes. De behandeling bestaat uit een dieet en leefstijl adviezen en indien dit onvoldoende effect heeft, wordt insuline therapie gestart. Onderzoek heeft aangetoond dat behandeling van zwangerschapsdiabetes een vermindering van het aantal ernstige complicaties tijdens de zwangerschap geeft.

Er zijn nog vele vraagstukken op het gebied van zwangerschapsdiabetes onbeantwoord o.a. met betrekking tot de pathofysiologie, effectieve preventie, monitoren van behandeling en lange-termijn follow-up. Zo is er nog geen adequate test die vroeg in de zwangerschap kan voorspellen of een zwangere vrouw diabetes gravidarum gaat ontwikkelen. Onderzoek naar biomarkers die detecteerbaar zijn vóórdat er sprake is van verhoogde bloedglucosewaarden, zou de identificatie van hoog risico zwangeren kunnen verbeteren. Hierdoor zouden preventieve maatregelen toegepast kunnen worden en behandeling in een vroeg stadium toegepast kunnen worden.

Daarnaast is momenteel ook veel (inter)nationale discussie omtrent de implementatie van de "International Association of Diabetes and Pregnancy Study Groups" (IADPSG) diagnostische criteria voor zwangerschapsdiabetes. Deze strengere criteria zijn aangenomen door verschillende internationale organisaties maar nog niet in Nederland. In het kader daarvan hebben wij onderzoek gedaan naar de zwangerschapsuitkomsten van vrouwen die zijn gescreend en behandeld volgens de huidige richtlijn "Diabetes en zwangerschap" van de Nederlandse Verneging voor Obstetrie en Gynaecologie.

Er zijn vele successen geboekt op het gebied van behandeling maar veel minder aandacht is er tot nog toe geweest voor de lange termijn follow-up na de zwangerschap. Volgens de richtlijn dienen vrouwen jaarlijks gescreend te worden op diabetes mellitus type 2 gedurende vijf jaar na de bevallig. Of deze aanbevelingen worden opgevolgd in de Nederlandse praktijk was niet bekend.

Het doel van dit proefschrift was om de klinische uitkomsten en biochemische aspecten van zwangerschapsdiabetes te evalueren; van biomarkers & risicostratificatie naar evaluatie van de behandeling, en lange-termijn follow-up.

In **deel I** van dit proefschrift bestudeerden wij biomarkers en hun rol in het voorspellen van zwangerschapsdiabetes.

Hoofdstuk 1 geeft een algemene inleiding en beschrijft de doelen van dit proefschrift.

Hoofdstuk 2 geeft een overzicht van de huidige literatuur met betrekking tot biomarkers bij en hun mogelijke rol in het voorspellen van zwangerschapsdiabetes. Laaggradige ontsteking en insuline resistentie is verergerd bij zwangerschapsdiabetes. Onderzoek heeft aangetoond dat bij vrouwen met zwangerschapsdiabetes er sprake is van een down-regulatie van adiponectine en anti-inflammatoire cytokines (adiponectin, interleukine-10) en een up-regulatie van leptine en inflammatoire cytokines (interleukine-6, tumor necrosis factor- alpha). Het toevoegen van biomarkers aan klinische predictiemodellen zou het identificeren van hoog risico zwangeren kunnen verbeteren. Momenteel bestuderen wij de relatie tussen adiponectine en zwangerschapsdiabetes en gaan we de voorspellende waarde onderzoeken van adiponectine in combinatie met een klinisch predictiemodel.

In **hoofdstuk 3** onderzochten we de rol van ghreline als biomarker bij zwangerschapsdiabetes. Behoudens het groeihormoonstimulerend effect heeft ghreline ook een belangrijke rol bij de glucosehomeostase. Onze hypothese was dat vrouwen met zwangerschapsdiabetes meer insuline-resistentie en hyperinsulinisme hebben, en daarom lagere ghrelin concentraties dan vrouwen zonder zwangerschapsdiabetes. Er werden ghrelin concentraties bij vrouwen met en zonder zwangerschapsdiabetes gemeten met behulp van een dubbele anti-lichaam sandwich ELISA kit. De resultaten toonde het tegenovergestelde, ghrelin concentraties waren vergelijkbaar in beide groepen. Daarnaast werd er een fysiologische daling gezien in ghreline concentraties na inname van 75-g glucose oplossing in beide groepen. Dit onderzoek toont aan dat ghreline vooralsnog geen geschikte biomarker is in zwangerschapsdiabetes.

In **deel II** evalueren wij de huidige behandeling van zwangerschapsdiabetes door middel van onderzoek naar de zwangerschapsuitkomsten en de wijze van monitoren van de behandeling. Tevens onderzoeken wij het effect van metformine bij zwangere vrouwen met een hoog risico op zwangerschapsdiabetes.

In **hoofdstuk 4** onderzochten we de zwangerschapsuitkomsten van een grote cohort multi-etnische vrouwen die gescreend en behandeld waren voor zwangerschapsdiabetes in Rotterdam. De zwangerschapsuitkomsten werden vergeleken met zwangerschappen zonder zwanger-schapsdiabetes (en zonder diabetes mellitus type 1 en 2) verkregen via de stichting Perinatale Registratie Nederland. Onafhankelijke voorspellers voor het gebruik van insuline therapie waren: BMI ≥ 30, OGTT waarden (nuchter en 2 uur), diabetes gravidarum in de voorgeschiedenis en etniciteit (Mediterraan).
BMI en het gebruik van insuline therapie waren onafhankelijke voorspellers voor het optreden van een neonatale complicatie. Het percentage inleidingen van de bevalling was relatief hoog

maar dit leidde niet tot extra veel bevallingen met een keizersnede. Wellicht heeft dit er mede voor gezorgd dat er een opvallend laag aantal pasgeborenen was dat te zwaar was voor de duur van de zwangerschap. Wij concludeerden dat behandeling van zwangerschapsdiabetes volgens de huidige richtlijn resulteert in een laag aantal zwangerschapscomplicaties in vergelijking met zwangerschappen zonder diabetes.

De behandeling van zwangerschapsdiabetes heeft als doel om normale bloedglucosewaarden te bereiken. Inzicht in de glucoseregulatie is daarom essentieel. Momenteel geschied dit via een glucose dagcurve door middel van capillaire vingerprikmetingen. In **hoofdstuk 5** presenteren wij de resultaten van het onderzoek naar het monitoren van de behandeling van zwangerschapsdiabetes. We onderzochten de rol van "continuous glucose monitoring" (CGM) voor het aantonen van verhoogde bloedglucosewaarden (hyperglycemie) bij vrouwen die behandeld werden voor zwangerschapsdiabetes. CGM toonde hyperglycemieën aan, welke niet gedetecteerd waren door de standaard glucose dagcurve. De duur van de hyperglycemie was echter niet substantieel.

In **hoofdstuk 6** onderzochten wij het effect van metformine bij zwangere vrouwen met een verhoogd risico op zwangerschapsdiabetes. De hypothese was dat het gebruik van metformine vroeg in de zwangerschap een effect zou hebben op de incidentie van zwangerschapsdiabetes. Het benodigde aantal vrouwen wat nodig was om dit te kunnen onderzoeken werd niet gehaald. Desalniettemin, werden er geen nadelige effecten van het gebruik van metformine in de zwangerschap geobserveerd. Bewijs dat het gebruik van metformine tijdens de zwangerschap geen nadelige lange-termijn effecten bij kinderen heeft is van essentieel belang.

In het **deel III** van dit proefschrift werd gekeken naar de lange-termijn follow-up van vrouwen met zwangerschapsdiabetes.

In **hoofdstuk 7** presenteren wij de resultaten van een onderzoek naar de screening van diabetes mellitus type 2 in de vijf jaar na de bevalling. We toonden aan dat maar ongeveer één derde van de vrouwen jaarlijks gescreend werd in de vijf jaar na de zwangerschap. Na het sturen van een reminder naar de huisartsen verdubbelde het screeningspercentage bijna. Wij concludeerden dat de huidige aanbevelingen voor diabetes screening suboptimaal worden nageleefd. Screening strategieën dienen worden te herzien en verbeterd.

In de discussie gaan we in op de interpretatie van de belangrijkste bevindingen van dit proefschrift en de implicaties voor de praktijk (**hoofdstuk 8**). Tevens geven wij aanbevelingen voor vervolgonderzoek.

In dit proefschrift laten we zien dat ghreline geen geschikte biomarker bij zwangerschapsdiabetes is. De behandeling van vrouwen met zwangerschapsdiabetes volgens de huidige nationale richtlijn is succesvol in het verlagen van het aantal complicaties bij moeder en kind. Continue glucosemonitoring heeft een beperkte meerwaarde in de detectie van hyperglycemieën vergeleken met een standaard glucose dagcurve bij vrouwen die behandeld worden voor zwangerschapsdiabetes. Metformine bij zwangere vrouwen met hoog risico op zwangerschapsdiabetes had geen effect op de incidentie van zwangerschapsdiabetes maar onderzoek met een groter aantal proefpersonen is noodzakelijk. Wel werden er geen ernstige nadelige korte termijn effecten van metformine geobserveerd. Verder hebben wij aangetoond dat de huidige aanbevelingen voor lange-termijn diabetes screening niet goed worden nageleefd en voor verbetering vatbaar zijn.

PORTFOLIO

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Maasstad Hospital Department: Internal Medicine	**Promotor: Prof.dr. A.J. van der Lelij**
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1. PhD training	**Year**	**Workload ECTS**
General courses		
Biomedical English Writing and Communication	2017	4
Basiscursus klinisch onderzoekers (BROK®)	2017	2
Epidemiology course – Maasstad Hospital	2017	1
E-Learning Biostatistics	2015	2
Good Clinical Practice – Maasstad Hospital	2015	1
Seminars and workshops		
Clinical Research Seminar, FDIME, Paris	2015	2
Post-DOC network: conflict management workshop	2016	0.5
Medical Business Masterclass, Amsterdam	2016	0.5
Medtronic, insulin pump therapy workshop	2016	0.8
ROIG, Diabetes in pregnancy, Erasmus MC	2016	0.2
Department journal & research club	2015-2017	1
Internal Medicine Department Education	2015–2017	2
Presentations		
Diabetes mellitus and interculturalisation, De Kuip, Rotterdam	2017	1
Dutch Endocrine Meeting, Noordwijkerhout	2016	2
Gestational diabetes: Who Cares? Symposium, De Kuip, Rotterdam	2016	2
SCEM symposium, Diabetes in pregnancy, Utrecht	2016	1
Wetenschapsdag, Maasstad Ziekenhuis (2 poster presentations, 1x oral presentation and award: best oral presentation)	2015–2017	3
Regional meeting: vascular medicine	2015	1
Annual meeting of the young investigators of the Dutch Association for Diabetes Research, Soesterberg	2015	1
EADV symposium, Utrecht	2015	1
Verloskundig Samenwerkingsverband (VSV), Maasstad Ziekenhuis	2015	1

	Year ECTS	Workload
International conferences		
International congress Obstetrics and Gynaecology, Prague (oral presentation)	2017	2
The 9th International symposium on Diabetes in Pregnancy, Barcelona (poster presentation)	2017	2
2. Teaching		
Clinical lessons to internal medicine nurses, Maasstad Hospital	2017	1
Diabetes in pregnancy, Department of Internal Medicine, Maasstad Hospital	2017	0.25
Gestational Diabetes, Department of Obstetrics and Gynaecology, Maasstad Hospital	2016	0.25
Supervising medical students: Larissa van der Houwen and Ankie van Damme	2015	1
Other skills		
Gestational Diabetes outpatient clinic	2015–2017	5
Role as sub-investigator in diabetes studies: – Study of MiniMed™ 640G Insulin Pump with SmartGuard™ in prevention of Low Glucose Events in adults with Type 1 diabetes. – Optimising patient relevant outcomes with Toujeo (insulin) glargine 300U/ml in routing diabetes care. – A multi-centre, prospective, non-interventional study of insulin degludec investigating the safety and effectiveness in a real world population with type 1 and type 2 diabetes mellitus.	2015–2017	5
Organizational skills		
Diabetes and interculturalisation – Rotterdam	2017	2
Diabetes gravidarum: Who Cares? Symposium – Rotterdam	2016	2

ABOUT THE AUTHOR

Huguette was born on March 4th 1988, in Hilversum, The Netherlands. In 2000, she moved with her parents and sister to South Africa to start on a great new adventure. She attended High School at Penryn College in Nelspruit, Mpumalanga and became Head of the Students' Representative Council. She became interested in medicine through public health projects in local townships. She graduated with academic honours and returned to the Netherlands in 2007 to start medical school at the University of Maastricht. She joined the student association and became president in 2010. After completing her Bachelor's degree in Medicine it was once again time to broaden her horizon and she moved to California for an internship at Stanford University Medical Center in California, USA. In February 2011 she continued medical school and completed several internships abroad (Belgium, Australia and Indonesia). After graduating she started working as a resident at the Department of Internal Medicine at the Maasstad Hospital in Rotterdam. In 2015 she became a PhD candidate under supervision of dr. J. van der Linden and professor A.J. van der Lelij, which resulted in this thesis. During her PhD she organised a symposium entitled "Gestational Diabetes: Who Cares?" At the end of her PhD she was happily accepted for specialty training in Internal Medicine in Rotterdam and she started this new chapter in January 2018.

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