Efforts to improve efficacy of screening for gestational diabetes mellitus

Ph.D. Thesis

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2005

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List of publications related to the subject of this thesis

I. **Bitó, T.**, Nyári, T., Kovács, L., Pál, A.: Oral glucose tolerance testing at gestational weeks ≤16 could predict or exclude subsequent gestational diabetes mellitus during the current pregnancy in high risk group. Eur J Obstet Gynecol Reprod Biol, in press (IF=1.002)

II. **Bitó, T.**, Földesi, I., Nyári, T., Pál, A.: Prediction of gestational diabetes mellitus in high risk group by insulin measurement in early pregnancy. Diabetic Medicine, in press (IF=2.235)

III. **Bitó, T.**, Kozinszky, Z., Pál, A.: Postpartum evaluation of hemoglobin A_{1c} level in mothers of ≥4000 g neonates: a screening method for gestational diabetes mellitus unrecognized antepartum. Diabetes Research and Clinical Practice, in press (IF=1.680)

IV. **Bitó, T.**, Nyári, T., Pál, A.: A 32-34. terhességi hétre manifesztálódó terhességi cukorbetegség előrejelzése a 24-28. terhességi héten végzett cukorterheléssel. Magy Nőorv L, in press

Abbreviations

ACOG:	American College of Obstetricians and Gynecologists
ADA:	American Diabetes Association
ANOVA:	analysis of variance
AUC:	area under the curve
BMI:	body mass index
CH:	carbohydrate
CI:	confidence interval
DM:	diabetes mellitus
EDTA:	ethylenediaminetetraacetic acid
g:	gram
GDM:	gestational diabetes mellitus
GOD-POD:	glucose oxidase-peroxidase
HbA_{1c}:	glycosylated haemoglobin
HPL:	human placental lactogen
HPLC:	high-pressure liquid chromatography
HSOG:	Hungarian Society of Obstetrics and Gynaecology
IFG:	impaired fasting glucose
IGT:	impaired glucose tolerance
NDDG:	National Diabetes Data Group
OGTT:	oral glucose tolerance test
OR:	odds ratio
RCT:	randomized controlled trial
RF:	risk factor
ROC:	receiver operating characteristic
SPSS:	Statistical Package for Social Sciences
STATA:	Statistical Software Package for Professionals
TNF-alpha:	tumour necrosis factor-alpha
WHO:	World Health Organization

CONTENTS

1. INTRODUCTION

Gestational diabetes mellitus (GDM) was initially described in 1964 by O'Sullivan and Mahan who documented the association between the results of an oral glucose tolerance test (OGTT) during pregnancy and the future development of type 2 diabetes mellitus (DM) (1). Screening and diagnostic procedures were based on this recognition and not on the perinatal outcome from the beginning. The relationship between carbohydrate (CH) intolerance in pregnancy and an adverse perinatal outcome is presumed to be a continuous one, and thus no single cut-off can divide pregnant women into those at high risk and those not at risk at all (2). Furthermore, there are no data to prove that the diagnosis and treatment of GDM will prevent or even delay the onset of future DM in the mother or her offspring (3). In the opinion of many experts, GDM is not a disease, but rather a concept relating to the disordered maternal metabolism and to its effect on the foetal physiology, growth and development (2,4). Thus, GDM should be regarded as a risk factor (RF) for several complications during pregnancy and for subsequent DM in the mother and her offspring.

GDM has been characterized as the onset or first recognition of glucose intolerance of variable severity during the current pregnancy (5-10). As concerns this definition, it is obvious that GDM is a heterogeneous entity which includes both a pregnancy-induced glucose intolerance ("true GDM"), type 1 DM with onset during pregnancy and an undiagnosed alteration of the CH metabolism (impaired glucose tolerance /IGT/ and subclinical type 2 DM) discovered during pregnancy (10-13). Changes in hormonal status, insulin resistance, food intake and physical activity result in an approximately 2-3-fold increase in insulin requirement during pregnancy, starting from gestational weeks 16 to 18 and increasing with gestational age (14). These physiological changes lead to GDM when the insulin requirement exceeds its secretion.

Although GDM can develop at any time during pregnancy (depending on the compensatory capacity of the pregnant woman), it is manifested most often in the third trimester (10,15,16) as the insulin requirement is highest at that time (15) due to the state of progressive glucose intolerance as gestation advances (17,18).

GDM is one of the most common complications during pregnancy: its reported prevalence varies from 1 to 14%, with 2 to 5% being the most common figure (19,20). In Hungary, the estimated number of new GDM cases is 2000 to 3000 per year (4,21). The incidence of GDM is highly population-specific, however; the number of detected GDM cases also depends on the screening method and the timing of screening (15,22,23).

Traditionally, the universal screening of all pregnant women was recommended by the American Diabetes Association (ADA). In 1986, the American College of Obstetricians and Gynecologists (ACOG) recommended selective screening for pregnant women at high risk of GDM. In 1994, the ACOG made no definite recommendation because of the absence of data supporting screening. The current recommendation of the ADA states that it is probably not cost-effective to screen patients at low risk of GDM. This low-risk group was defined as women meeting all of the following criteria: age <25 years, normal body weight (body mass index /BMI/ <27 kg/m^2), no first-degree relatives with DM, and not a member of a racial or ethnic group with a high prevalence of type 2 DM. There are at least two arguments which support universal screening: approximately 50% of patients with GDM have no RF at all (24,25), and using the selective screening guideline of the ADA's recent recommendation excludes only 10% of the population, though only 3% of the GDM cases would have been missed (26).

The most commonly used screening and diagnostic methods are the two-step method satisfying the National Diabetes Data Group (NDDG) criteria (27) or its modification by Carpenter and Coustan (28), and the one-step method according to the WHO criteria (29,30). The attractiveness of the WHO criteria is that it is an easy method, acceptable for pregnant women, and it is comparable to the postpartum OGTT (3). The two-step method with a random 50-g glucose load as a screening test, and in the event of a positive test, a 100-g OGTT as a diagnostic test is less convenient for pregnant women and not comparable to the method of postpartum reclassification of the CH metabolism; however, it is closer to the original method of O'Sullivan and Mahan. In Hungary, the screening method recommended by the WHO has come into general use, but the technical bulletin of the Hungarian Society of Obstetrics and Gynaecology (HSOG) published in 1992 accepts both the one-step and two-step methods (25). General screening for GDM for all pregnant women with a 75-g OGTT at gestational weeks 24 to 28 is recommended by the Hungarian Diabetes Association according to the WHO recommendation (29,30). Those pregnant women with RFs for GDM, e.g. a family history of DM, a history of an adverse perinatal outcome (macrosomia, malformation, polyhydramnios, stillbirth or missed abortion), a maternal age >35 years, obesity, hypertension or glucosuria, are recommended to undergo screening in the prepregnant state (in the event of a planned pregnancy) or at the first prenatal visit in the first trimester (11,12,31). This will identify women with previously undiagnosed DM to whom appropriate counselling, diagnostic procedures and treatment may be offered (31-35). Subsequent testing

is recommended at gestational weeks 24 to 28 if the screening in early pregnancy yielded a normal result.

Despite 40 years of research, there is still a lack of consensus regarding nearly every clinical aspect of GDM: the need to screen, the diagnostic criteria, the treatment and even the validity of GDM as a meaningful diagnosis (36). Currently, there is no "gold standard" for the diagnosis of GDM and hence the need for randomized controlled trials (RCTs) has become even more important (3). However, such a RCT could be at least questionable for ethical considerations. There are still many questions concerning the screening and diagnosis of GDM: who to screen, how to screen, when to screen, and what glucose level should considered to be the limit between normal and pathological.

From cost-benefit considerations, there is no consensus even concerning the need to screen. As "true GDM" is usually a glucose intolerance of moderate severity with late onset, it hardly influences the foetal physiology, growth and development, whereas pregestationally existing alterations in CH metabolism discovered during pregnancy can lead to a number of serious complications. Thus, the definition of GDM should be revised to presumable pregestational and to pregnancy-induced alterations in CH metabolism. The detection of patients in the first group is important as concerns the influence on the perinatal outcome, maternal complications during pregnancy and future DM in the mother and her offspring, while the detection of pregnant women with "true GDM" is important for the likelihood of subsequent DM later in life in both the mother and her infant. Screening earlier would result in a lower number of cases, as it would detect merely those with severe or presumable pregestational alterations in CH metabolism. However, because of the early detection and management, serious complications could be prevented. Screening later in pregnancy would result in a higher number of cases, with detection of moderate disturbances of the CH metabolism (pregnancy-induced glucose intolerance) too, but serious complications could hardly be prevented due to the late recognition.

GDM can remain unrecognized in the event of a concealed pregnancy, use of an inadequate method and/or the unsuitable timing of screening, or when the disturbance of the CH metabolism is only moderate and occurs in the third trimester, after the GDM screening procedure has been performed.

The most common complication of GDM is macrosomia of the neonate, which is 3 to 4 times more likely to occur in a pregnancy complicated by GDM (37). A neonatal birthweight of $\geq$4000 g occurs in 25% to 42% of diabetic pregnancies (38). In the event of a macrosomic neonate, the question arises of whether the unrecognized GDM is responsible for

the increased birthweight. The maternal CH metabolism should be examined again despite a previous negative OGTT in the event of a macrosomic neonate. The postpartum OGTT appears to be unreliable to determine whether the unrecognized GDM is responsible for the increased birthweight (≥4000 g) (39). A possible reason for this might be the change in the CH metabolism and insulin sensitivity in the early postpartum period, due to the rapid decrease of placental hormones. Glycosylated haemoglobin (HbA_{1c}) is an indicator of the long-term glycaemic status, reflecting the average blood glucose level of the last 4-6 weeks (40,41). Thus, HbA_{1c} reflects the maternal glucose metabolism retrospectively in the early puerperium. There are few data in the Medline database on the postpartum evaluation of the maternal CH metabolism, and only one evaluation involved the postpartum HbA_{1c} (39). Recognition of women with antecedent GDM is important for the management of their next pregnancy; furthermore, these women carry a very high lifetime risk of developing type 2 DM (42).

2. OBJECTIVES

A method for the screening and diagnostics of GDM should meet two requirements: 1) to detect as far as possible all GDM cases, and 2) to achieve the early detection of the cases so as to prevent complications. It seems obvious that there is no ideal screening and diagnostic method, and also that one screening test during pregnancy may not be sufficient for both requirements in some cases.

We set out to develop a screening and diagnostic protocol for GDM with the highest possible sensitivity and predictivity and with the earliest possible detection of CH alterations in pregnant women at high risk of GDM. For this reason, we sought a suitable protocol to meet both requirements.

2.1. Efforts to detect all GDM cases

We presumed that HbA_{1c} evaluation in the early postpartum period in cases of mothers with ≥4000 g neonates could help in the detection of undiagnosed GDM cases at risk of future type 2 DM. To detect those pregnant women with GDM whose altered CH metabolism was undetectable at gestational weeks 24 to 28, it seems logical to perform an additional screening test at gestational weeks 32 to 34. Thus, we could probably prevent a macrosomia of the neonate by adequate management in previously undetected cases. However, it could be expensive and unnecessary to repeat the GDM screening in all pregnant women with a negative screening result at gestational weeks 24 to 28. It would be useful to identify cut-off values for fasting and 120-min serum glucose levels of the OGTT at gestational weeks 24 to 28, whereby subsequent alterations in CH metabolism could be excluded or predicted in order to decrease the population undergoing repeated screening.

2.2. Efforts to detect alterations as early as possible

We presumed that negative OGTTs with glucose values close to the generally accepted cut-off levels of IGT reflect borderline cases which could convert to GDM as gestation advances. We hypothesized that an OGTT result close to these cut-off values at screening in early pregnancy can predict GDM. The relationship between the 2-hour, 75-g OGTTs at gestational weeks ≤16, 24 to 28 and 32 to 34 has previously not been addressed.

It seems logical to perform insulin evaluation before gestational weeks 16 to 18 too, as the insulin level does not rise until this gestational age due to pregnancy-induced changes; thus alterations in serum glucose and insulin levels reflect the presumable pregestational, but previously undetected cases. Thus, we could attain the earliest detection of altered cases. A negative OGTT with an increased fasting and/or postprandial serum insulin level reflects an increased demand on the compensatory capacity of the pregnant woman. We hypothesized that an increased serum insulin level at screening in early pregnancy can predict GDM.

2.3. Additional objective

From cost-benefit considerations and to make the screening protocol easier and more applicable, we wished to reduce the number of screening examinations to a minimum which would still satisfy our double requirement of early and total detection.

2.4. Aims of the present study

1. To establish an optimum screening protocol (close to the ideal one) as concerns the demand for the earliest detection, and the ideal sensitivity and specificity of the method.
2. To examine whether HbA_{1c} evaluation in the early postpartum period is a suitable method with which to detect antepartum undiagnosed GDM cases in the event of an increased neonatal birthweight (≥4000 g).
3. To determine the proportion of women with an elevated postpartum HbA_{1c} level (unrecognized GDM cases) in the population of women with neonates of increased birthweight (≥4000 g).
4. To detect factors possibly predictive of an elevated postpartum maternal HbA_{1c} level.
5. To determine those fasting and/or 120-min glucose levels of OGTT at gestational weeks 24 to 28 under which the further testing could be omitted as the subsequent manifestation of GDM is very unlikely.
6. To determine those fasting and/or 120-min glucose levels of OGTT at gestational weeks 24 to 28 above which further testing should not be recommended as the subsequent manifestation of GDM is very likely.
7. To determine the proportion of the population for whom further testing should be recommended at gestational weeks 32 to 34.

8. To identify the cut-off fasting and postload glucose levels at gestational weeks ≤16 in a high-risk group, below which no further OGTT is necessary as the possibility of GDM is excluded.
9. To determine the cut-off fasting and postload glucose levels at gestational weeks ≤16 in a high-risk group, above which no further OGTT is necessary as the subsequent manifestation of GDM is strongly predicted.
10. To assess the proportion of the group at high risk of GDM who could be spared any subsequent OGTT by application of these cut-off values.
11. To determine the predictive values of the different RFs for GDM at gestational weeks 24 to 28 and 32 to 34.
12. To determine the positive and negative predictive values of increased serum insulin levels at fasting and/or postload at gestational weeks ≤16 for GDM at gestational weeks 24 to 28 in a high-risk group.
13. To determine the positive and negative predictive values of increased serum insulin levels at fasting and/or postload at gestational weeks ≤16 for GDM at gestational weeks 32 to 34 in a high-risk group.

3. MATERIALS AND METHODS

3.1. Study patients

3.1.1. Postpartum evaluation of HbA_{1c} level in mothers of ≥4000 g neonates

This prospective observational study was carried out between 1 May 1995 and 31 Oct 1999. During this period, there were 7912 singleton deliveries in our Department. All Caucasian subjects (n=610) with singleton pregnancies who had given negative screening results for GDM previously and who had delivered neonates in our Department with birthweights ≥4000 g in gestational weeks 37 to 41 without appreciable blood loss in connection with the delivery (postpartum haematocrit and/or haemoglobin less than 30 vol% and 10 g/dl, respectively, on postpartum day 3) were enrolled in the study. Screening for GDM was not performed or the result was not accessible in 247 of the 7912 cases. These 247 women were not enrolled in the study, and thus 7665 cases remained. GDM was diagnosed by OGTT in 192 of these singleton cases (2.5%). The neonatal birthweight was ≥4000 g in 637 (8.5%) of the 7473 non-GDM cases. The control group was recruited from volunteer women who delivered their neonates with birthweights of 2500-3990 g in gestational weeks 37 to 41 in our Department on the same days, and who had also given negative screening results for GDM in gestational weeks 24 to 28. Twenty-seven of the 637 women in the study group and 33 of the 568 recruited controls were excluded from further analysis as they had a low haematocrit and/or haemoglobin level on postpartum day 3. Accordingly, 610 and 535 women remained in the study and the control group, respectively.

3.1.2. Prediction of GDM in a high-risk group by insulin measurement in early pregnancy

This prospective observational study was carried out between 1 Jan 2001 and 28 Feb 2002. All pregnant women referred to our special Diabetic Pregnant Outpatient Department who displayed one or more RFs for GDM (n=90) were enrolled in the study. Nineteen of the 90 patients were excluded from the study as they were referred to our Department after gestational week 16 or had had GDM in a previous pregnancy. The pregnant women who had had GDM in a previous pregnancy were managed as patients with pregestational DM. After their informed consent had been given, 71 pregnant women before gestational weeks 16 were

scheduled for a 2-hour, 75-g OGTT according to the WHO criteria (29,30), with serum insulin determination both at fasting and at 2 hours. Seven patients were excluded from the further analysis as GDM was diagnosed in this first OGTT before gestational week 16.

Subsequent OGTTs without insulin determination were performed in the remaining 64 pregnant women at gestational weeks 24 to 28 and, for those with a normal result (n=48), at gestational weeks 32 to 34.

3.1.3. Prediction of GDM in a high-risk group by OGTT in early pregnancy

This prospective observational study was carried out between 1 Jan 2001 and 30 Sept 2002. All pregnant women who had not had GDM in a previous pregnancy or any alteration in CH metabolism in their history, but who displayed one or more RFs for GDM and who had been referred to our special Diabetic Pregnant Outpatient Department (n=163) at gestational weeks ≤16 were enrolled in the study. These pregnant women were not opposed to any medication or dietary restriction. After their informed consent had been given, the pregnant women were scheduled for a 2-hour, 75-g OGTT according to the WHO criteria at gestational weeks ≤16 (29,30). Eight patients were excluded from the further analysis as GDM was diagnosed by this first OGTT at gestational weeks ≤16. Subsequent OGTTs were performed in the remaining 155 pregnant women at gestational weeks 24-28 and, for those with a normal result (n=123), at gestational weeks 32-34.

3.1.4. Prediction of GDM at gestational weeks 32 to 34 by OGTT performed at gestational weeks 24 to 28

This prospective observational study was carried out between 1 Jan 2002 and 31 Dec 2002. All pregnant women (n=149) whose screening for GDM was performed in our special Diabetic Pregnant Outpatient Department by 75-g OGTT at gestational weeks 24 to 28 according to the WHO recommendations (29,30) were enrolled in the study after giving their informed consent. GDM was diagnosed in 24 of the 149 pregnant women, and they were excluded from further analysis. For those with a negative result (n=125), subsequent testing was performed at gestational weeks 32 to 34.

The sensitivity and specificity of the fasting and postload glucose levels at gestational weeks 24 to 28 to predict subsequent GDM at gestational weeks 32 to 34 were examined in the ranges 5.0 to 6.9 mmol/l and 6.0 to 7.7 mmol/l for the fasting and the 2-hour

postload, respectively, in 0.1 mmol/l steps. False-positive and false-negative ratios of ≤10% were considered to be acceptable.

3.2. Screening for GDM

In order to diagnose GDM, pregnant women were scheduled for a 2-hour, 75-g OGTT at gestational weeks 24 to 28 in accordance with the WHO recommendation (29,30). The pregnant women were instructed to consume at least 150 g CH/day for 3 days, and then to fast overnight for 10 to 12 hours on the day before the test. Plasma samples were obtained for glucose evaluation by repeated venipuncture at fasting and 120 min after ingestion of a 75-g glucose solution over 5 min. Pregnant women were considered to have GDM in the event of a glucose level of ≥7.0 mmol/l at fasting and/or of ≥7.8 mmol/l at 120 min, according to the WHO criteria. Subsequent OGTT was performed at gestational weeks 32 to 34 in the event of pregnant women with a normal result.

Those women with one or more RFs for GDM, such as a family history of diabetes, a history of an adverse perinatal outcome (macrosomia, malformation, polyhydramnios, stillbirth or missed abortion), a maternal age ≥35 years, obesity, hypertension or glucosuria, were screened at the first prenatal visit, and subsequent testing was performed at gestational weeks 24 to 28 if the screening in early pregnancy had yielded a normal result. Those pregnant women who had had GDM in a previous pregnancy were managed as subjects with pregestational DM.

3.3. Assessment of RFs for GDM

The incidences of the following RFs for GDM were analysed in these studies: any family history of type 2 DM, a history of a large neonate (≥4000 g), a history of an adverse perinatal outcome (missed abortion, malformation, polyhydramnios, stillbirth or preterm delivery), obesity (a prepregnant BMI (weight(kg)/height^2(m^2)) ≥30), age ≥35 years and glucosuria.

Analysis of maternal and neonatal morphometric data

The maternal BMI was calculated and analysed by means of the WHO/NIH classification of overweight and obesity (43,44). The prepregnant weight or that at the first pregnant care visit was used for calculation of the maternal BMI.

The neonatal weight was measured immediately after delivery.

3.5. Methods of HbA_{1c}, plasma glucose and serum insulin evaluation

3.5.1. HbA_{1c} evaluation

HbA_{1c} levels were determined on the fasting blood samples of the 75-g OGTT and in the event of a neonatal birthweight of $\geq$4000 g within 72 hours after delivery. HbA_{1c} was assayed on ethylenediaminetetraacetic acid (EDTA)-mediated blood by high-pressure liquid chromatography (HPLC) (before 2000) or by microparticle enzyme immunoassay (Abbott Laboratories, IL, USA) (from 2000), with an interassay coefficient of variation of 6.4% and an intraassay coefficient of variation of 4.4%. A HbA_{1c} level $\geq$6.0% was considered to be elevated.

3.5.2. Plasma glucose evaluation

Glucose levels were determined by the GOD-POD (glucose oxidase-peroxidase) colorimetric method (Randox Laboratories Ltd., Crumlin, UK) on sodium fluoride-mediated blood obtained by venipuncture. Both the interassay and the intraassay coefficient of variation were <2%.

3.5.3. Serum insulin evaluation

Serum insulin levels were determined by chemiluminescent immunoassay (DPC Immulite 1000, Diagnostic Products Co. Los Angeles, CA, USA) on native blood obtained by venipuncture, with an interassay coefficient of variation of 7.6% and an intraassay coefficient of variation of 4.8%. This method has a cross-reaction with proinsulin of 8.5% and has no cross-reaction with C-peptide and glucagons, as stated in the original description of the method.

Serum insulin levels of ≥30 mU/l at fasting and ≥70 mU/l at 120 min were considered to be hyperinsulinaemic, based on our laboratory reference ranges for the population of BMI ≥27, which are similar to the values given in the protocol description of Immulite and to that described by Ascaso et al. in the obese non-pregnant population (45) with a normal glucose metabolism.

3.6. Statistical analysis

All data are presented as means ± SD. Statistical significance was set at the 95% level ($p < 0.05$).

3.6.1. Postpartum evaluation of HbA_{1c} level in mothers of ≥4000 g neonates

Two-way analysis of variance (ANOVA) was used to compare the different groups, with Bonferroni pairwise correction. Receiver operating characteristic (ROC) curve analysis was performed to determine the best threshold for neonatal birthweight and the maternal BMI to study the possibility of predicting an elevated HbA_{1c} level. Statistical analyses were performed with SPSS for Windows, version 9.0 (SPSS, Chicago, IL, USA).

3.6.2. Prediction of GDM in a high-risk group by insulin measurement in early pregnancy

The ANOVA and multiple logistic regression methods were carried out for statistical analysis using the Stata Software Package (StataCorp LP, College Station, TX, USA). The Hosmer-Lemeshow goodness-of-fit test was performed to check the models. The sensitivity, the specificity, and the positive and negative predictive values of the fasting and postload insulin levels were calculated for an assessment of the possibility of predicting GDM at gestational weeks 24 to 28 and 32 to 34.

3.6.3. Prediction of GDM in a high-risk group by OGTT in early pregnancy

Statistical analyses were carried out with the Stata Software Package (StataCorp LP, College Station, TX, USA). Logistic regression analyses were performed via a ROC plot to determine cut-off values for the best accuracy of diagnosis. The Hosmer-Lemeshow

goodness-of-fit test was performed to check the models (46). The sensitivity, the specificity, and the positive and negative predictive values of the fasting and postload glucose levels were calculated for an assessment of the possibility of predicting GDM at gestational weeks 24 to 28 and 32 to 34. To evaluate the relative risk of GDM at gestational weeks 24 to 28 and 32 to 34, the odds ratios (ORs) of the best cut-off values of the fasting and 120-min glucose values of the OGTT at gestational weeks $\leq$16 and of the different RFs were calculated.

3.6.4. Prediction of GDM at gestational weeks 32 to 34 by OGTT performed at gestational weeks 24 to 28

Statistical analyses were carried out with the Stata Software Package (StataCorp LP, College Station, TX, USA). The Student test, ANOVA and logistic regression analyses were performed.

4. RESULTS

4.1. Postpartum evaluation of HbA_{1c} level in mothers of ≥4000 g neonates

An elevated level was found in 60 (9.8%) of the 610 cases in the study group and in 11 (2.1%) of the 535 controls ($p<0.001$), although the mean postpartum HbA_{1c} level was not significantly different in the two groups. Table 1 shows the demographic and morphometric data and postpartum HbA_{1c} levels in the study and control groups. The mean gestational age at delivery was significantly ($p<0.001$) lower in the control group than in the study group (38.2±2.2 vs. 39.5±1.1 gestational weeks, respectively). The mean maternal BMI in the study group was significantly ($p<0.001$) higher than that in the control group (29.5±4.6 vs. 27.2±4.0, respectively).

Table 1. Demographic data, maternal BMI, neonatal birthweight and postpartum HbA_{1c} levels for the study population.

Variables	Mothers with neonates of birthweight ≥4000 g	Controls	Significance
No.	610	535	
Mean age (years)	28.5 ± 5.2	27.3 ± 5.2	p<0.001
Mean gestational age at delivery (weeks)	39.5 ± 1.1	38.2 ± 2.2	p<0.001
Mean maternal BMI (kg/m^2)	29.5 ± 4.6	27.2 ± 4.0	p<0.001
Male:female ratio	1.85	1.62	NS
Mean neonatal birthweight (g)	4211 ± 219	3199 ± 489	p<0.001
Mean postpartum HbA_{1c} (%)	5.3 ± 0.6	5.3 ± 0.5	NS
No. (%) of cases with elevated postpartum HbA_{1c} level	60 (9.8)	11 (2.1)	p<0.001

NS: non-significant

The study group was next divided into two subgroups on the basis of the postpartum HbA_{1c} level: subgroup A: women with HbA_{1c} <6.0%, and subgroup B: women with HbA_{1c} ≥6.0% (Table 2). The women in subgroup A had a significantly ($p<0.01$) higher mean gestational age at delivery, a significantly ($p<0.01$) lower maternal BMI and a significantly ($p<0.01$) lower neonatal birthweight than those for the women with an elevated HbA_{1c} level. There was no significant difference between these subgroups as concerns the maternal age or the neonatal male:female ratio.

Table 2. Subgroups of mothers with neonates of birthweight ≥4000 g on the basis of the postpartum HbA_{1c} level.

Variables	Subgroup A: HbA_{1c} <6%	Subgroup B: HbA_{1c} ≥6%	Significance
No.	550	60	
Mean neonatal birthweight (g)	4203 ± 228	4291 ± 278	p<0.01
Mean maternal BMI (kg/m^2)	29.3 ± 4.4	31.8 ± 5.4	p<0.001
Mean gestational age at delivery (weeks)	39.5 ± 1.0	39.0 ± 1.6	p<0.01
Mean age (years)	28.2 ± 5.1	28.7 ± 5.5	NS
Male:female ratio	1.86	1.48	NS

NS: non-significant

The study group was divided into 3 subgroups on the basis of the neonatal birthweight (4000-4499 g, 4500-4999 g and ≥5000 g). The subgroup with a birthweight of 4000-4499 g involved 90% of the cases. A significant difference was not found between the subgroups in the case of mean postpartum HbA_{1c} level. The maternal BMI increased significantly with the neonatal birthweight (Table 3).

Table 3. Subgroups of the study group on the basis of the neonatal birthweight.

Variables	Neonatal birthweight (g)		
	4000-4499	4500-4999	≥5000
No. (%)	549 (90)	56 (9.2)	5 (0.8)
Mean age (years)	28.5 ± 5.2	28.6 ± 5.4	27.4 ± 4.1
Mean gestational age at delivery (weeks)	39.5 ± 1.1	39.5 ± 1.5	39.6 ± 0.6
Mean maternal BMI (kg/m^2)	29.4 ± 4.5	31.0 ± 5.4 **	32.6 ± 3.4 ***
Mean postpartum HbA_{1c} (%)	5.3 ± 0.6	5.4 ± 0.6	5.5 ± 1.0

Difference from the 4000-4499 g birthweight subgroup: *: p< 0.05, **: p<0.01, ***: p<0.001

The study group was divided into 4 subgroups on the basis of the maternal BMI. Both the neonatal birthweight and the postpartum maternal HbA_{1c} levels increased slightly with the maternal BMI (Table 4). Overweight and obesity occurred in 84.3% of those in the study group, while 95% of the women with elevated HbA_{1c} levels in the study group were overweight or obese. The proportion of cases with elevated HbA_{1c} levels increased markedly with increase in the maternal BMI.

ROC curve analysis was utilized to determine the best threshold of the neonatal birthweight and maternal BMI with which to predict an elevated postpartum HbA_{1c} level. The best threshold for the neonatal birthweight with which to predict an elevated postpartum maternal HbA_{1c} level was as low as 3695 g, with a prediction sensitivity of 89.3% and a specificity of 42.1%. The area under the curve (AUC) was 0.687. The threshold of a 4000 g neonatal birthweight yielded a sensitivity of 83.9% and a specificity of 48.3%. The sensitivity

decreased to 70.9% and the specificity increased to 60.6% when the 90th birthweight percentiles were used. The best threshold for the prediction of an elevated HbA_{1c} was at a maternal BMI of 27.3, with a sensitivity of 75.5% and a specificity of 47.1%. The AUC was 0.645.

Table 4. Subgroups of the study group on the basis of the maternal BMI, using the WHO classifications of overweight and obesity.

Variables	Maternal BMI (kg/m^2)			
	≤ 24.9 Normal weight	25.0 - 29.9 Overweight	30.0 - 34.9 Moderate obesity	≥ 35.0 Serious obesity
No. (%)	96 (15.7)	278 (45.6)	168 (27.5)	68 (11.1)
Mean age (years)	27.6 ± 3.8	27.9 ± 1.4	29.6 ± 5.3 **	29.3 ± 5.8 *
Mean gestational age at delivery (weeks)	39.6 ± 0.9	39.5 ± 0.1	39.5 ± 1.3	39.4 ± 1.2
Mean neonatal birthweight (g)	4176 ± 194	4195 ± 181	4247 ± 273 *	4258 ± 246 *
Mean postpartum HbA_{1c} level (%)	5.1 ± 0.5	5.2 ± 0.5	5.4 ± 0.6 ***	5.5 ± 0.6 ***
No. (%) of cases with elevated postpartum HbA_{1c} level	3 (3.1%)	23 (8.3%)	18 (10.7%)*	16 (23.5%)***
% of all cases with elevated postpartum HbA_{1c}	5	38.3***	30***	26.7**

Difference from the subgroup with normal weight: *: $p< 0.05$ **: $p<0.01$ ***: $p<0.001$

There were a total of 252 GDM cases (192 diagnosed antepartum and 60 postpartum), which means that only 76.2% of the GDM cases were recognized antepartum when the screening method recommended by WHO was used (29,30).

4.2. Prediction of GDM in a high-risk group by insulin measurement in early pregnancy

GDM was diagnosed in 43 (60.5%) of the 71 pregnant women with one or more RFs for GDM who were referred to our Department at gestational weeks ≤16: in 7 (16.3%) in the first OGTT before gestational week 16, in 13 (30.2%) at gestational weeks 24 to 28, and in 23 (53.5%) at gestational weeks 32 to 34. There were GDM totals of 7, 20 and 43 by gestational weeks 16, 24 to 28 and 32 to 34, respectively.

Table 5. Demographic, morphometric and metabolic parameters (at gestational weeks ≤ 16) of subgroups of pregnant women on the basis of the onset of GDM.

Variables	Onset of GDM			Non-GDM
	at gw ≤16	at gw 24 to 28	at gw 32 to 34	
No. of cases	7	13	23	28
Mean age (years)	27.3 ± 5.1	29.8 ± 5.3	29.0 ± 6.0	28.9 ± 5.8
Mean BMI (kg/m^2)	27.1 ± 6.6	32.0 ± 7.5*	29.5 ± 6.4	28.2 ± 5.3
Mean HbA_{1c} level (%)	7.7 ± 2.2***×##	6.2 ± 0.9	6.0 ± 0.5	5.7 ± 0.5
Mean glucose (mmol/l)				
at fasting	5.8 ± 1.7***×	5.4 ± 0.7***	4.9 ± 0.5	4.6 ± 0.4
at 120 min	8.8 ± 1.2***×××##	7.1 ± 0.4***	6.2 ± 1.2	5.6 ± 1.0
Mean insulin (mU/l)				
at fasting	31.8 ± 9.0***××	32.2 ± 6.6***×××	21.5 ± 8.0	16.1 ± 6.3
at 120 min	96.5 ± 11.6***×	94.7 ± 22.7***××	69.0 ± 24.0**	47.4 ± 22.4
No. of cases with 1 RF	4	7	14	19
No. of cases with ≥2RFs	3	6	9	9

gw: gestational weeks
RF: risk factor
Significance levels were calculated using the Bonferroni method.
Difference from the non-GDM group: *:p<0.05 **:p<0.01 ***:p<0.001
Difference from the group with GDM at gw 32 to 34: ×:p<0.05 ××:p<0.01 ×××:p<0.001
Difference from the group with GDM at gw 24 to 28: #:p<0.05 ##:p<0.01 ###:p<0.001

The pregnant women were divided into subgroups on the basis of the gestational age at the onset of GDM (Table 5). The HbA_{1c}, plasma glucose and insulin levels both at fasting and at 120 min decreased with increase in gestational age at the diagnosis of GDM.

The incidence of subsequent GDM was analysed in pregnant women with a negative result (n=64) on the first OGTT at gestational weeks ≤16. The pregnant women were divided into 3 subgroups on the basis of the fasting and 120-min serum insulin levels at gestational weeks ≤16: normal at both fasting and 120 min, normal at fasting but increased at 120 min, and both increased (Table 6).

No case was found with increased fasting, but normal 120-min serum insulin levels. In the subgroup with increased serum insulin levels both at fasting and at 120 min, GDM occurred in 9 of the 13 cases at gestational weeks 24 to 28, and GDM was not manifested at all in only 1 of the 13 cases. GDM was manifested at gestational weeks 24 to 28 in only 3 of the 18 cases with normal fasting and increased 120-min serum insulin levels, and in 12 of the 18 cases at gestational weeks 32 to 34. Of the 33 cases with normal fasting and 120-min

serum insulin levels, 24 were non-GDM. GDM was manifested in 1 of the 33 cases at gestational weeks 24 to 28, and in 8 cases at gestational weeks 32 to 34.

Table 6. Age, BMI, HbA_{1c}, and glucose levels of subgroups of pregnant women on the basis of the serum insulin level at gestational weeks ≤16.

Variables	Serum insulin level (mU/l) at gw ≤16		
	≥30 at fasting ≥70 at 120 min	<30 at fasting ≥70 at 120 min	<30 at fasting <70 at 120 min
No. of cases	13	18	33
- GDM at gw 24 to 28	9	3	1
- GDM at gw 32 to 34	3	12	8
- non-GDM cases	1	3	24
Mean age (years)	30.0 ± 5.2	29.2 ± 5.6	28.8 ± 6.1
Mean BMI (kg/m^2)	32.4 ± 7.3*	30.2 ± 6.5	27.9 ± 5.3
Mean glucose (mmol/l)			
at fasting	5.2 ± 0.8*	5.0 ± 0.6	4.7 ± 0.4
at 120 min	6.8 ± 0.8**	6.6 ± 1.0**	5.6 ± 1.1
Mean HbA_{1c} level (%)	6.1 ± 1.0	6.0 ± 0.4	5.9 ± 0.6
No. of cases with 1 RF	6 (46.2%)	10 (55.6%)	24 (72.7%)
No. of cases with ≥2 RFs	7 (53.8%)	8 (44.4%)	9 (27.3%)

RF: risk factor
gw: gestational weeks
Significance levels were calculated using the Bonferroni method.
Difference from the group with serum insulin levels <30 mU/l and <70 mU/l at fasting and at 120 min, respectively, at gw ≤16: *:$p<0.05$ **:$p<0.01$ ***:$p<0.001$

The sensitivity, the specificity, and the positive and negative predictive values of increased fasting or 120-min serum insulin levels for the prediction of a glucose intolerance by gestational weeks 24 to 28 and 32 to 34 are shown in Table 7. An increased fasting serum insulin level had a higher positive predictive value as compared with an increased 120-min insulin level at gestational weeks 24 to 28 and 32 to 34. Higher negative predictive values for gestational weeks 24 to 28 and 32 to 34 were found in the event of an increased serum insulin level at 120 min than at fasting.

The sensitivity, the specificity, and the positive and negative predictive values of increased fasting serum insulin levels for the prediction of an impaired fasting glucose (IFG) by gestational weeks 24 to 28 and 32 to 34 were also calculated. An increased fasting serum insulin level (≥30 mU/l) predicted IFG at gestational weeks 24 to 28 with a sensitivity of 94%, a specificity of 50%, a positive predictive value of 0.37 and a negative predictive value of 0.96. An increased fasting serum insulin level (≥30 mU/l) predicted IFG at gestational weeks 32 to 34 with a sensitivity of 76%, a specificity of 87%, a positive predictive value of 0.65 and a negative predictive value of 0.92.

Table 7. Sensitivity, specificity, and positive and negative predictive values of serum insulin levels at fasting and at 120 min before gestational week 16 for the prediction of glucose intolerance by gestational weeks 24 to 28 and 32 to 34.

Variables	Increased serum insulin level at gw ≤ 16			
	at fasting (≥30 mU/l)		at 120 min (≥70 mU/l)	
	GDM by gw			
	24 to 28	32 to 34	24 to 28	32 to 34
Sensitivity	69.2%	33.3%	92.3%	75.0%
Specificity	96.4%	96.4%	85.7%	85.7%
Positive predictive value	0.9	0.92	0.75	0.87
Negative predictive value	0.87	0.53	0.96	0.73

gw: gestational weeks

The incidence data relating to the examined RFs for GDM were as follows: a family history of DM: 49.3% (35 out of 71), obesity (prepregnant BMI ≥30): 46.5% (33 out of 71), age ≥35 years: 21.1% (15 out of 71), glucosuria: 15.5% (11 out of 71), a history of a large neonate (≥4000 g): 9.9% (7 out of 71), a history of an adverse perinatal outcome (including missed abortion, malformation or stillbirth): 5.6% (4 out of 71). There was more than one RF for GDM in 27 (38.0%) of the 71 cases.

Univariate analysis showed that the fasting and 120-min plasma glucose levels, HbA_{1c}, BMI, and the fasting serum insulin level significantly predicted GDM at 24 to 28 and 32 to 34 weeks with an OR range of 1.4 to 31.0 and wide confidence intervals (CI), except for BMI for GDM at gestational weeks 32 to 34. The plasma glucose levels and serum insulin levels at fasting and at 120 min, HbA_{1c}, and BMI were used in the multiple logistic regression analysis. An increased serum insulin level at fasting (OR: 16.6, 95%; CI: 2.06–134.2) was the best predictor of GDM at gestational weeks 24 to 28 in the multiple logistic regression model. Additionally, an increased serum insulin level at 120 min (OR: 13.3, 95% CI: 3.07–57.9) was the best predictor of GDM by gestational weeks 32 to 34 in the multiple logistic regression model. An increased HbA_{1c} was associated non-significantly with GDM at gestational weeks 24 to 28 (OR: 4.3, 95% CI: 0.67–27.8) and 32 to 34 (OR: 1.6, 95% CI: 0.44–5.53). The other factors (the BMI, and the plasma glucose levels at fasting and 120 min) were non-significant in the multiple regression model. All the models gave a good fit.

4.3. Prediction of GDM in a high-risk group by OGTT in early pregnancy

GDM was diagnosed in 88 (54.0%) of the 163 pregnant women at high risk of GDM who were referred to our Department at gestational weeks ≤16: in 8 (4.9%), 32 (19.6%) and

48 (29.4%) by the OGTT at gestational weeks ≤16, 24 to 28 and 32 to 34, respectively. There were GDM totals of 8, 40 and 88 by gestational weeks 16, 24 to 28 and 32 to 34, respectively. Those 8 women who met the criteria of GDM by the first OGTT at gestational weeks ≤16 were excluded from the study. The remaining 155 pregnant women were enrolled in the study.

The pregnant women were divided into subgroups on the basis of the gestational age at the onset of GDM. The age, the BMI, the glucose levels at fasting and at postload and the frequency of RFs are shown in Table 8. There were no differences in mean age between the subgroups except between the subgroups of non-GDM pregnant women and those with GDM at gestational weeks 24 to 28 ($p<0.05$). Both subgroups of pregnant women with GDM at gestational weeks 24 to 28 and 32 to 34 had a significantly ($p<0.01$) higher mean BMI than that of those in the non-GDM subgroup: 28.4±7.3, 27.8±5.9 and 25.3±4.4, respectively. The mean glucose levels both at fasting and at postload of the OGTT at gestational weeks ≤16 decreased significantly with increase in gestational age at the diagnosis of GDM; the lowest levels were found in the non-GDM subgroup. The proportions of pregnant women with more than one RF were 40.6%, 37.5% and 20% in the subgroups of pregnant women with GDM at gestational weeks 24 to 28 or 32 to 34 and the non-GDM subgroup, respectively.

Table 8. Age, BMI, glucose levels at fasting and at 120-min postload at gestational weeks ≤16 and distributions for one or more RFs among the study population (n=155).

Variables	Onset of GDM		Non-GDM	Total
	at gw 24 to 28	at gw 32 to 34		
No. of cases	32	48	75	155
Mean age (years)	30.2 ± 4.9*	28.6 ± 5.3	28.1 ± 5.3	28.7 ± 5.2
Mean BMI (kg/m^2)	28.4 ± 7.3**	27.8 ± 5.9**	25.3 ± 4.4	26.7 ± 5.6
Mean glucose (mmol/l)				
at fasting	5.4 ± 0.7***†	4.9 ± 0.5**	4.6 ± 0.4	4.9 ± 0.6
at 120 min	7.1 ± 0.4***††	6.2 ± 1.2*	5.5 ± 1.0	6.1 ± 1.1
No. of cases with 1 RF	19 (59.4%)	30 (62.5%)	60 (80%)	109 (70.3%)
No. of cases with ≥2 RFs	13 (40.6%)	18 (37.5%)	15 (20%)	46 (29.7%)

RF: risk factor
gw: gestational weeks
Difference from the non-GDM group: *:$p<0.05$ **:$p<0.01$ ***:$p<0.001$
Difference from the group with GDM at gw 32 to 34: †:$p<0.05$ ††:$p<0.01$ †††:$p<0.001$

The best cut-off value for the fasting glucose level was 5.0 mmol/l, with negative predictive values of 0.92 and 0.61 at gestational weeks 24 to 28 and 32 to 34, respectively. Approximately a quarter (24.5%) of the pregnant women at high risk had a fasting glucose

level under this cut-off value. Those pregnant women with a fasting glucose level ≥5 mmol/l were at significantly higher risk (OR: 3.8, 95% CI: 1.1-13.4]) of subsequent GDM at gestational weeks 24 to 28 than were those with a fasting glucose level <5 mmol/l. However, the relevant relative risk of GDM at gestational weeks 32 to 34 was non-significant: OR: 1.9, 95% CI: 0.9-4.0.

The best cut-off value for the postload glucose level was 6.2 mmol/l, with negative predictive values of 1.00 and 0.70 at gestational weeks 24 to 28 and 32 to 34, respectively. Merely 15% of the pregnant women at high risk had a postload glucose level under this cut-off value. Those pregnant women with a postload glucose level ≥6.2 mmol/l were at significantly higher risk of subsequent GDM at gestational weeks 24 to 28 (OR: 7.5, 95% CI: 1.0-57.8) and 32 to 34 (OR: 2.6, 95% CI: 1.1-6.5) than were those with a postload glucose level <6.2 mmol/l.

The best cut-off values for the combination of fasting and postload glucose levels were 5.3 mmol/l for the fasting level and 6.8 mmol/l for the postload level, with negative predictive values of 0.97 and 0.71 and sensitivities of 96.9 and 86.3 at gestational weeks 24 to 28 and 32 to 34, respectively. The false-positive ratios were 73.5% and 41.0% at gestational weeks 24 to 28 and 32 to 34, respectively. Approximately a quarter (24.5%) of the pregnant women at high risk had glucose levels under these cut-off values. The sensitivity, the specificity, the positive and negative predictive values and the best cut-off values for the fasting and postload glucose levels are presented in Table 9.

Table 9. Diagnostic characteristics of the best cut-off values for fasting and/or postload glucose levels to predict subsequent GDM.

Variables	Best cut-off level at fasting: 5.0 mmol/l		Best cut-off level at 120 min: 6.2 mmol/l		Best combined cut-off levels: 5.3 mmol/l at fasting 6.8 mmol/l at 120min	
	GDM at gestational weeks					
	24 to 28	32 to 34	24 to 28	32 to 34	24 to 28	32 to 34
Sensitivity	90.6	81.3	100.0	91.3	96.9	86.3
Specificity	28.5	30.7	18.7	21.3	30.1	36.0
Positive predictive value	0.25	0.56	0.24	0.55	0.26	0.59
Negative predictive value	0.92	0.61	1.00	0.70	0.97	0.71

The relative risks of GDM at gestational weeks 24 to 28 and 32 to 34 for the most frequently occurring RFs and their distribution are shown in Table 10. Obesity proved to be the strongest RF for GDM at gestational weeks 32 to 34, with an OR of 3.31, 95% CI: 1.32-8.29. Other RFs were not significant as concerns the 95% CIs. A family history of DM and obesity occurred most frequently: in 33.8% and 31.5%, respectively, of the pregnant women at high risk. The occurrence of an adverse perinatal outcome and a large neonate (≥4000 g) was 3.8% and 4.6%, respectively.

Table 10. Relative risks of GDM at gestational weeks 24 to 28 and 32 to 34 for the most frequent RFs and their distribution.

RFs	% of all RFs	GDM at gw 24 to 28 OR [95% CI]	GDM at gw 34-32 OR [95% CI]
Family history of DM	33.8	0.41 [0.13-1.27]	0.67 [0.28-1.58]
Obesity	31.5	2.39 [0.82-6.92]	3.31 [1.32-8.29]
Age ≥35 years	16.9	1.63 [0.53-5.02]	1.36 [0.50-3.70]
Glucosuria	9.2	1.29 [0.24-7.01]	1.22 [0.27-5.48]

RF: risk factor
gw: gestational weeks

In combination, the best cut-off values for fasting (5.3 mmol/l) and postload (6.8 mmol/l) with obesity proved to be very strong predictive factors for GDM by gestational weeks 32 to 34, with an OR of 6.0, 95% CI: 1.7-21.0. The area under the ROC curve was 0.82 (Hosmer-Lemeshow goodness-of-fit test: p=0.31).

4.4. Prediction of GDM at gestational weeks 32 to 34 by OGTT performed at gestational weeks 24 to 28

Characteristics of the studied population are shown in Table 11. There was no significant difference in mean age between the pregnant women with and without GDM at gestational weeks 32 to 34. The mean BMI was significantly ($p<0.05$) higher in the pregnant women with GDM than in those without GDM (27.8 ± 0.8 kg/m^2 vs 25.5 ± 0.5 kg/m^2, respectively). The plasma glucose levels at gestational weeks 24 to 28 both at fasting and at 120 min were significantly higher ($p<0.001$ for both) for those with subsequent GDM than for those without it.

Table 11. Characteristics of the study population.

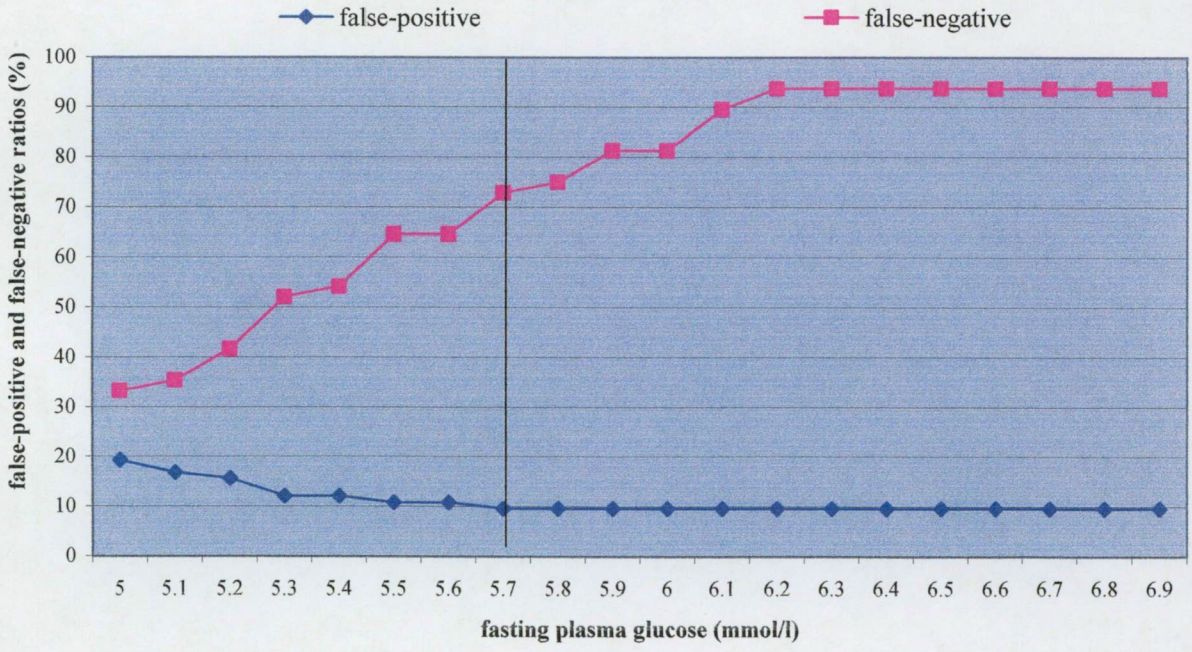
Variables	GDM at gw 32 to 34 (n= 48)	Non-GDM at gw 32 to 34 (n= 77)	Total (n=125)	Difference between GDM and non-GDM cases
Mean age (years)	28.6 ± 5.3	28.0 ± 5.2	28.2 ± 5.2	p=0.548
Mean BMI	27.8 ± 5.9	25.4 ± 4.6	26.3 ± 5.2	p=0.014
Mean plasma glucose level at gw 24 to 28:				
at fasting	5.2 ± 0.6	4.5 ± 0.4	4.7 ± 0.6	p<0.001
at 120 min	7.1 ± 0.7	5.8 ± 1.2	6.3 ± 1.2	p<0.001

gw: gestational weeks

False-positive and false-negative ratios of the different fasting plasma glucose levels as cut-off values for the prediction of subsequent GDM are shown in Figure 1.

The subsequent manifestation of GDM at gestational weeks 32 to 34 was found to be very likely in the event of a fasting plasma glucose level of ≥5.7 mmol/l (indicated with a line in Figure 1): false-positive ratio: 9.6%, sensitivity: 0.27, specificity: 0.90. Merely 16% of the examined population had a fasting plasma glucose level ≥5.7 mmol/l.

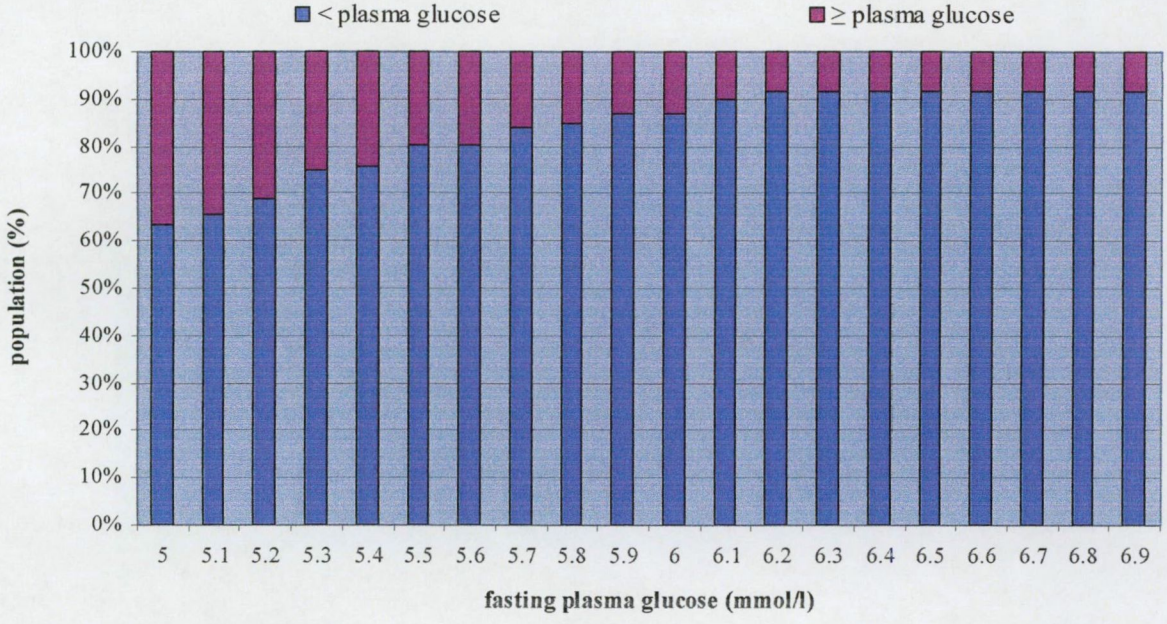
Figure 1. False-positive and false-negative ratios of different fasting plasma glucose levels at gestational weeks 24 to 28 for prediction of GDM at gestational weeks 32 to 34



The false-positive ratio <10% in the event of a fasting plasma glucose ≥5.7 mmol/l (denoted by a vertical line in the Figure).

The proportions of the population with plasma glucose levels lower than or equal to or higher than the given cut-off values are shown in Figure 2. We failed to identify a fasting plasma glucose cut-off value in the examined range under which subsequent manifestation of GDM would be so unlikely that further testing could be omitted. The false-negative ratio was higher than 10% even when a fasting plasma glucose level of 5.0 mmol/l was applied as a cut-off value.

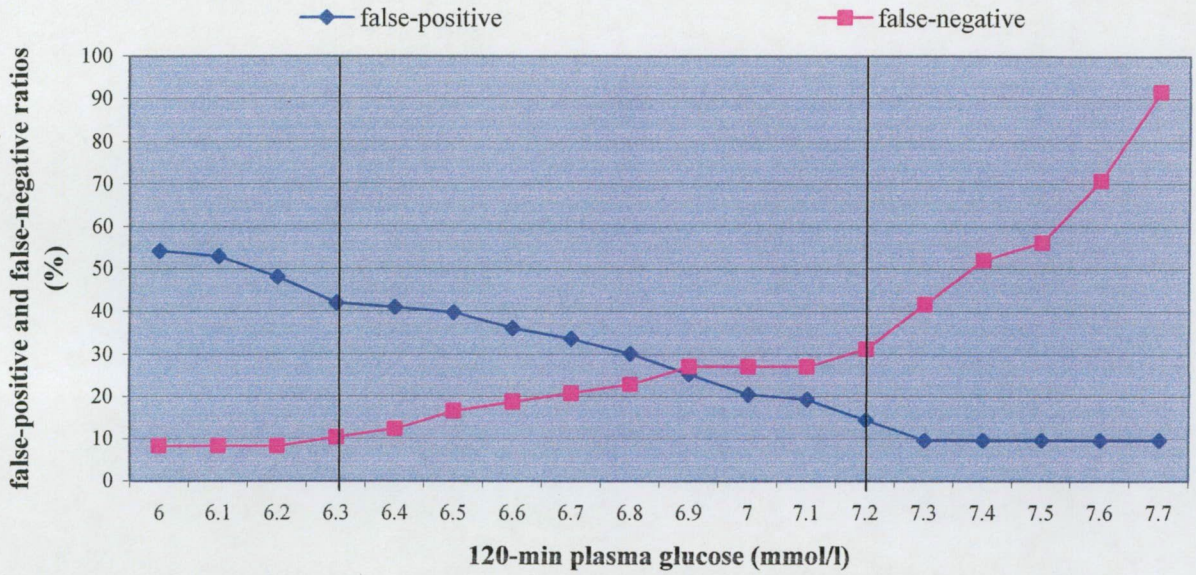
Figure 2. Distribution of population on the basis of the fasting plasma glucose



False-positive and false-negative ratios of the different plasma glucose levels at 120 min as cut-off values for the prediction of subsequent GDM are shown in Figure 3.

The subsequent manifestation of GDM at gestational weeks 32 to 34 was found to be very unlikely in the event of a plasma glucose level of <6.3 mmol/l at 120 min (false-negative ratio: 8.3%). As many as 35.9% of the population met this criterion. The subsequent manifestation of GDM at gestational weeks 32 to 34 was found to be very likely in the event of a plasma glucose level of ≥7.3 mmol/l at 120 min (false-positive ratio: 9.6%, sensitivity: 0.58, specificity: 0.90), while 27.5% of the study population met this criterion.

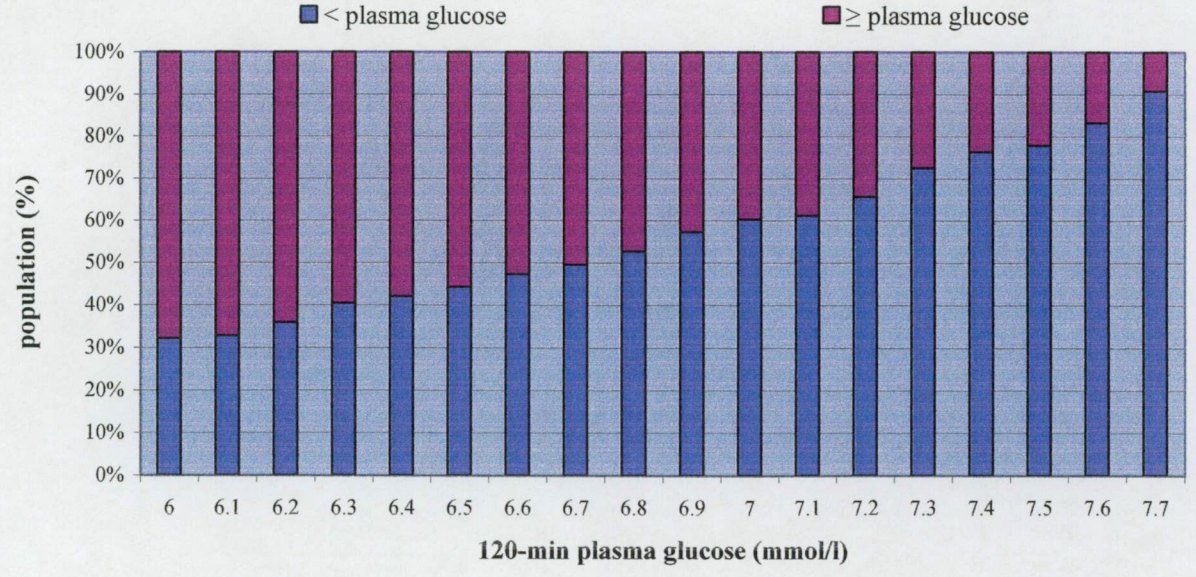
Figure 3. False-positive and false-negative ratios of different 120-min plasma glucose levels at gestation weeks 24 to 28 for prediction of GDM at gestational weeks 32 to 34



Subsequent OGTT is recommended at gestational weeks 32 to 34 between the two vertical lines.

The proportions of the population with plasma glucose levels lower than or equal to or higher than the given cut-off values are shown in Figure 4. If subsequent screening for GDM were performed merely in the event of a plasma glucose level of between 6.3 and 7.2 mmol/l at 120 min, the further OGTT could be omitted in 63.4% of the population.

Figure 4. Distribution of population on the basis of the 120-min plasma glucose



5. DISCUSSION

5.1. Postpartum evaluation of HbA_{1c} level in mothers of ≥4000 g neonates

Various authors have examined the CH metabolism in cases involving an increased neonatal birthweight. The postpartum 100-g OGTT has been proposed by Carpenter et al. to detect antecedent GDM in women whose diagnoses have been missed, but who have the relevant RFs (47), and by Bukulmez et al. for mothers of macrosomic infants (48). According to the postpartum testing criterion proposed by Carpenter et al., antecedent GDM may be predicted with a sensitivity of 80% and a specificity of 90% (47); while Bukulmez et al. found the same sensitivity (80%), but a lower specificity (78%) (48). We consider that the postpartum OGTT is unsuitable for this purpose, in consequence of the rapid decrease in insulin requirement and change in insulin sensitivity after delivery. The maternal HbA_{1c} level in the early postpartum period reflects the average glucose level of the last 4 to 6 weeks of gestation, which appears to make this method applicable for the detection of antecedent alterations in the CH metabolism. Kurishita et al. performed a 75-g OGTT and HbA_{1c} examination within the first 3 days postpartum in 59 women who gave b irth to heavy-for-dates infants (exceeding the mean + 1.5 SD of the Japanese foetal growth curve chart (49)), and found that OGTTs are unreliable during the puerperium to reflect the maternal glucose metabolism retrospectively, while an elevated HbA_{1c} level of the dense erythrocytes in the postpartum implies a subtle hyperglycaemic status in late pregnancy (40). We performed HbA_{1c} evaluation in a Caucasian population with larger case numbers. We consider that women with infants with a neonatal birthweight of ≥4000 g and an elevated postpartum HbA_{1c} level should be strongly suspected of having GDM which was unrecognized during pregnancy. In our study, the postpartum HbA_{1c} examination predicted antecedent GDM with a sensitivity of 83.9% and a specificity of 48.3%.

Macrosomia is defined as a birthweight above the 90th centile (50,51). In the present study, neonates with birthweights of ≥4000 g were analysed. The advantage of the method applied is that the use of different male and female centiles is unnecessary. Screening by postpartum HbA_{1c} examination for antenatally unrecognized GDM in the case of a birthweight of ≥4000 g displayed a better sensitivity and a poorer specificity than for the 90th birthweight centile. Use of a higher neonatal birthweight threshold would not lead to better sensitivity and specificity of the method.

Schaefer-Graf et al. found that maternal obesity, but not maternal glucose values correlated best with high rates of fetal macrosomia in pregnancies complicated by GDM (**52**). They defined fetal macrosomia as an abdominal circumference ≥90th centile by ultrasound examinations and maternal obesity as BMI ≥30 kg/m^2. In our study, the maternal BMI increased significantly with increasing neonatal birthweight (Table 3). This supports the observation of Schaefer-Graf et al. that maternal obesity appears to be a strong RF for macrosomia throughout pregnancies with GDM (**52**). In our study, although 95% of the cases with an elevated HbA_{1c} level were associated with the overweight or obesity of the pregnant women, this hardly helps us to decrease the population for screening via the postpartum HbA_{1c} level as 84.3% of the mothers with neonates with birthweights ≥4000 g were overweight or obese (Table 4). The mean postpartum HbA_{1c} level did not differ in the women with a normal maternal BMI and in the overweight subgroup (maternal BMI: 25.0-29.9) as only a small percentage of the cases had an elevated postpartum HbA_{1c} level. The proportion of women with an elevated HbA_{1c} level increased markedly as the maternal BMI rose, but it was "only" 23.5% in the most obese subgroup (Table 4). It is our opinion that using a neonatal birthweight of 3695 g, and a maternal BMI of 27.3 as optimum thresholds resulted in ROC curves that are too low, which could lead to an increased population for screening with a poorer cost:benefit ratio.

In our study, 23.8% of the GDM cases were not recognized during pregnancy, although we performed universal screening at gestational weeks 24 to 28 according to the WHO recommendation (29,30). As the subjects with an elevated HbA_{1c} level in the study group displayed a pregnancy-induced glucose intolerance with late onset, the glucose intolerance can hardly be expected to persist 6 to 8 weeks postpartum. Hence, alterations in the maternal CH metabolism should be evaluated in the early puerperium by HbA_{1c} examination, which we consider to be the only suitable method for this purpose. We presume that these women are also at increased risk of overt DM later in life. For this reason, these women should undergo repeated evaluations of the CH metabolism.

5.2. Prediction of GDM in a high-risk group by insulin measurement in early pregnancy

The increase in the insulin level starts at gestational weeks 16 to 18 (14). Hyperinsulinaemia after this gestational age reflects an approximately 2-3-fold increase in

insulin level, induced by pregnancy. Hyperinsulinaemia before this gestational age demonstrates that the patient is really hyperinsulinaemic, independently of the pregnancy.

Hyperinsulinaemia is a significant RF forDM. We consider hyperinsulinaemia in the non-pregnant state or before gestational weeks 16 to 18 to be a RF for GDM as it means insulin resistance. We hypothesized that hyperinsulinaemia in early pregnancy merely requires time to transform to GDM.

To date, few studies have dealt with the insulin level with a view to the prediction of insulin resistance or GDM. Several authors have examined the insulin level during and after pregnancy in patients with GDM in order to be able to predict the development of DM (53,54). However, we have found no prospective evaluation of the fasting insulin level or the insulin response to a 75-g OGTT in early pregnancy to predict a subsequent GDM during the ongoing pregnancy.

Ergin et al. examined the insulin response to a 100-g 3-hour OGTT in 120 Turkish women between 24 and 28 weeks of gestation (55). The fasting insulin level and insulin resistance did not differ in the patients with a single abnormal value of the OGTT and with GDM.

Kirwan et al. investigated the sensitivity indicated by an OGTT and also fasting glucose/insulin levels in an effort to predict insulin sensitivity in women before and during pregnancy (56). They repeated a 2-hour euglycaemic-hyperinsulinaemic clamp and a 120-min OGTT (a 75-g load in prepregnancy, and a 100-g load in pregnancy) on 15 women in prepregnancy and in both early (12 to 14 weeks) and late (34 to 36 weeks) pregnancy. They found that the insulin sensitivity indicated by the OGTT is significantly better than the fasting glucose and insulin values for the assessment of insulin sensitivity. However, the use of such an inconvenient method clearly limits the case number.

Clark et al. found that patients with GDM had higher insulin and C-peptide levels both at fasting and at 2 hours as compared with non-GDM patients (57). They determined insulin and C-peptide between 16 and 33 weeks of gestation, and found that these variables were predictive of GDM individually. They suggested that GDM should be looked upon as a component of the syndrome of insulin resistance.

Swinn et al. concluded that the excessive secretion of insulin precursors characterizes and predicts GDM (58). They examined the insulin, the intact proinsulin and the 32,33-split proinsulin response to an OGTT in 64 women with GDM and in 154 non-GDM control subjects of comparable age and BMI. The women with GDM were characterized by higher plasma insulin and intact proinsulin levels at 120 min and by elevated 32,33-split proinsulin

levels both at fasting and at 120 min. These insulin secretion abnormalities in GDM patients are similar to those seen in non-pregnant subjects with an impaired glucose tolerance. They also measured the insulin and proinsulin-like molecules in women with a 1-hour glucose level of > 7.7mmol/l after a 50-g glucose challenge at 28 to 32 weeks of gestation. The percentage of total insulin-like molecules accounted for by proinsulin-like molecules was significantly elevated in those women in whom a subsequent OGTT showed GDM versus those in whom the later OGTT was normal. To improve the predictive power of screening tests for GDM, Swinn et al. suggested the incorporation of a measurement of the percentage of proinsulin-like molecules in the routine 50-g screening test. In our view, the incorporation of serum insulin determinations at fasting and at 120 min in the screening protocol for GDM seems worthwhile and more applicable than the calculation of proportions or the use of expensive and inconvenient methods, but it is reasonable only in pregnant women with a RF for GDM before gestational week 16.

We found a positive correlation between the alteration in the serum insulin level and the subsequent manifestation of GDM. The more serious the alteration in the serum insulin level, the earlier the manifestation of GDM. A majority (83.3%) of the pregnant women with an elevated serum insulin level at 120 min subsequently manifested GDM by gestational weeks 32 to 34. GDM was manifested at gestational weeks 24 to 28 in 69.2% of those with elevated serum insulin levels both at fasting and at 120 min, and at gestational weeks 32 to 34 in 66.7% of those with a normal fasting level but an increased 120-min serum insulin level at ≤16 gestational weeks, but it was not manifested at all in 72.7% of those with normal serum insulin levels both at fasting and at 120 min at gestational weeks ≤16.

Increased serum insulin levels both at fasting and 120 min before gestational week 16 proved to be very strong predictive factors for GDM by gestational weeks 32 to 34, with an OR of 16.6 and 13.3, respectively.

As a fasting plasma glucose level of 5.6 mmol/l is the generally accepted cut-off value for IFG in pregnancy, we analysed fasting serum insulin levels in order to predict the possibility of subsequent IFG (59). We found that an increased fasting serum insulin level (≥30 mU/l) at gestational weeks ≤16 has very good sensitivity (94%) but poor specificity (50%) to predict IFG at gestational weeks 24 to 28, and the sensitivity was decreased to 76% while the specificity was increased to 87% concerning IFG at gestational weeks 32 to 34. The negative predictive values were very high at both gestational weeks 24 to 28 and 32 to 34. However, IFG does not mean GDM, and pregnant women with IFG should undergo a subsequent OGTT to evaluate the alterations in the CH metabolism.

Our method may reduce the number of subsequent screening tests for GDM, and in addition it facilitates optimization of the timing of the subsequent screening procedures. Furthermore, it can possibly prevent some complications of GDM, such as increased foetal growth, by earlier introduction of the appropriate management of the pregnant woman.

5.3. Prediction of GDM in a high-risk group by OGTT in early pregnancy

The identification and appropriate management of women with GDM improves the maternal, foetal and neonatal outcomes. Attention is currently focused on maximizing detection rates and diagnosing GDM as early as possible. First-trimester glucose screening is advantageous because patients with pre-existing DM are then identified as early as possible. Previously, others have examined the correlation between the 1-hour, 50-g glucose screening test and the 3-hour, 100-g OGTT in the different trimesters, but the relationship between the 2-hour, 75-g OGTTs at gestational weeks ≤16, 24 to 28 and 32 to 34 has previously not been addressed so far.

Nahum and Huffaker found a significant correlation between the results of first- and early third-trimester 1-hour, 50-g glucose screening tests (10). They concluded that third-trimester glucose screening may be unnecessary for patients with first-trimester glucose screening test values of ≤6.1 mmol/l (110 mg/dl), while for those with glucose values of >7.8 mmol/l (140 mg/dl) a direct third-trimester 3-hour OGTT is recommended.

Benjamin et al. examined 101 pregnant women from a high-risk population with a 1-hour, 50-g glucose screening test in the first trimester and 3-hour, 100-g OGTTs in the second and third trimesters (60). They recommended a third-trimester 3-hour, 100-g OGTT for all patients who gave positive screening tests even in the presence of normal follow-up second-trimester OGTTs.

We identified fasting (5.3 mmol/l) and postload (6.8 mmol/l) glucose cut-off values under which the subsequent manifestation of GDM at gestational weeks 24 to 28 is very unlikely (negative predictive value: 0.92), even in pregnant women at high risk of GDM. Hence, subsequent OGTT at gestational weeks 24 to 28, which is recommended by several authors and the WHO (29,30), does not appear reasonable. However, with regard to the lower negative predictive value (0.60) of these cut-off values for GDM at gestational weeks 32 to 34, subsequent OGTT should be performed at gestational weeks 32 to 34. One of the main goals of our method is that 24.5% of the pregnant women at high risk of GDM met this criterion.

On analysis of all combinations of different fasting and 120-min postload glucose cut-off values in steps of 0.1 mmol/l, the best (i.e. the lowest) false-positive ratios were 73.5% and 41.0% at gestational weeks 24 to 28 and 32 to 34, respectively, using 5.3 mmol/l and 6.8 mmol/l glucose levels for fasting and for postload, respectively. As concerns this very high false-positive ratio, we could not identify fasting and/or postload glucose cut-off values above which subsequent OGTT omission seems acceptable. Hence, for those at high risk and above these cut-off values, subsequent OGTT is recommended at gestational weeks 24 to 28. Identification of such upper cut-off values probably failed because of the relatively low number of cases in our study. It seems worthwhile to extend the number of cases so as to obtain precise upper cut-off values above which subsequent GDM could be strongly predicted at gestational weeks 16. The management of pregnant women with glucose levels above such cut-off values would be the same as for those with already confirmed GDM, as it merely takes time to convert to GDM. We could probably prevent complications related to GDM by early dietary management, particularly in obese pregnant women.

As concerns the examined RFs for GDM, none of them except obesity proved significant. Even obesity was non-significant for the prediction of GDM at gestational weeks 24 to 28. This means that RFs are useful to identify pregnant women at high risk of GDM, for whom OGTT is recommended in early pregnancy, but is not of predictive value for GDM.

5.4. Prediction of GDM at gestational weeks 32 to 34 by OGTT performed at gestational weeks 24 to 28

An increase in the production of hormones with an anti-insulin effect (particularly human placental lactogen (HPL) (61,62), cortisol (61), oestrogens (63) and progesterone (64)) and increasing levels of tumour necrosis factor-alpha (TNF-alpha) and leptin (65) lead to the demand for an increase in insulin secretion to maintain normoglycaemia as gestation advances. An increase in plasma glucose level due to the relative lack of insulin secretion despite hyperinsulinaemia occurs in women whose insulin demand exceeds their insulin secretion. GDM is diagnosed when the plasma glucose level attains or exceeds 7.0 mmol/l at fasting and/or $\geq$7.8 mmol/l at 2-hours by the 75-g OGTT (29,30). The timing of screening for GDM is influenced by two main requirements: 1) to detect as far as possible all GDM cases, and 2) to detect the alteration as early as possible so as to prevent maternal, foetal and neonatal complications by adequate management. One screening test during pregnancy does not seem to be sufficient for both requirements: screening earlier allows the earlier application

of adequate management with which foetal and maternal complications can be prevented, but decreases the number of detected cases; while screening later improves the detection rate, but the maternal and/or foetal complications can not be prevented in some cases. Screening at gestational weeks 24 to 28 is recommended by the WHO (29,30); however, the manifestation of the alteration in CH metabolism occurs later in many cases.

In this study, we tried to identify those cut-off levels of the OGTT at gestational weeks 24 to 28 with which the manifestation of GDM at gestational weeks 32 to 34 could be predicted or excluded. False-positive and false-negative rates of $\leq$10% were considered to be acceptable. A fasting plasma glucose level was found to be less useful than the 2-hour level for this purpose. It seems that the subsequent OGTT at gestational weeks 32 to 34 can be omissitted if the plasma glucose level is <6.3 mmol/l at 2 hours of the OGTT at gestational weeks 24 to 28, as the subsequent manifestation of GDM is then very unlikely. This means that subsequent OGTT can be omitted in 35.9% of the population. Subsequent OGTT is recommended merely if the ultrasound and/or other clinical alterations (i.e. polyhydramnios, macrosomia or glucosuria) relate to GDM. The subsequent manifestation of GDM at gestational weeks 32 to 34 is very likely in the event of a 2-hour plasma glucose of $\geq$7.3 mmol/l at gestational weeks 24 to 28. Thus, instead of subsequent testing, a quantitative diet is recommended in spite of the negative OGTT finding. A 2-hour plasma glucose level of $\geq$7.3 mmol/l at gestational weeks 24 to 28 occurred in 27.5% of the study population. Thus, it seems that subsequent testing can be omitted in 63.4% of the pregnant population. The goals of using these cut-off values are the preventive aspect, with less inconvenience for both the pregnant woman and the prenatal care service, and the cost-effectiveness. Subsequent testing at gestational weeks 32 to 34 is recommended merely in the event of a 2-hour plasma glucose level of between 6.3 and 7.2 mmol/l.

The explanation for the relative high cumulative prevalence of GDM at gestational weeks 24 to 28 (16.1%) and 32 to 34 (32.2%) could be the high rate of pregnant women at risk of GDM in the study population.

GDM can occur at early gestation due to the "diabetogenic effect" of the pregnancy in the event of an obese woman with insulin resistance and hyperinsulinaemia probably in the prepregnant state. A quantitative diet is recommended in the event of obesity, regardless of the result of the OGTT. Thus, a false-positive ratio of >10 % could be accepted in the event of an obese pregnant woman. Not merely GDM is responsible for macrosomia; it can also be caused by obesity (66) and hyperlipidaemia (particularly hypertriglyceridaemia) (67).

The avoidance of native glucose consumption and a quantitative diet can be recommended, in spite of an OGTT with a negative result, for pregnant women with BMI of 25 to 30 and of $\geq$30, respectively, but these pregnant women should not be considered to have GDM.

6. CONCLUSIONS

6.1. Postpartum evaluation of HbA_{1c} level in mothers of ≥4000 g neonates

Early postpartum maternal HbA_{1c} evaluation seems to be an easy method with acceptable efficacy for the diagnosis of previously unrecognized GDM in cases with a neonatal birthweight of ≥4000 g. However, this method is merely a retrospective screening tool, and thus does not improve the actual perinatal morbidity and mortality; though it may influence preparation for a future pregnancy, so it is still important to recognize antecedent GDM cases. We consider that these women require the same follow-up as for those with GDM diagnosed antepartum. Reclassification of the DM needs to be performed in postpartum week 6. Subsequent pregnancies of these women should be managed as pregestational DM; preconceptional care and adequate pregnancy care could then prevent foetal malformations and may improve the perinatal morbidity and mortality. Appropriate management of these women can postpone or prevent the manifestation of type 2 DM and diabetic complications, thereby providing them with a better quality of life and reduction of the health-care costs. This method draws attention to the insufficiencies and the efficacy of the screening method applied for GDM; furthermore, it may also be utilized for a comparison of the efficacies of different screening methods.

6.2. Prediction of GDM in a high-risk group by insulin measurement in early pregnancy

Fasting and postprandial serum insulin measurements at gestational weeks ≤16 seems to be an easy and reliable method with which to predict GDM in patients with a RF for GDM. With regard to the very high positive predictive value of the method at gestational weeks 32 to 34, pregnant women with elevated fasting and/or 120-min serum insulin levels at gestational weeks ≤16 should be managed in the same way as those with a diagnosis of glucose intolerance, in spite of a negative OGTT. As concerns the very high negative predictive value of the method at gestational weeks 24 to 28, pregnant women with normal serum insulin levels at fasting and at 120 min at gestational weeks ≤16 should undergo a subsequent OGTT merely at gestational weeks 32 to 34. A quantitative diet, independently of the results of the OGTT, might be considered for pregnant women with obesity, one of the most frequent RFs for GDM.

6.3. Prediction of GDM in a high-risk group by OGTT in early pregnancy

In conclusion, OGTT at gestational weeks ≤16 seems to be an obvious method with which to exclude the subsequent manifestation of GDM in pregnant women at high risk. Use of our cut-off values helps in the appropriate timing of the subsequent screening of this population. Required visits and further OGTTs can be reduced by utilizing this simple method in the screening for GDM, which makes the pregnancy care less inconvenient for the women, and reduces costs. Non-obese pregnant women with glucose levels under the cut-off values of 5.3 mmol/l at fasting and 6.8 mmol/l at 120-min postload should undergo subsequent testing merely at gestational weeks 32 to 34. We failed to identify a cut-off value above which subsequent GDM can be strongly predicted, due to the very high false-positive rate. However, the combination of the cut-off values for fasting (5.3 mmol/l) and postload (6.8 mmol/l) with obesity proved to possess a very strong predictive value for GDM by gestational weeks 32 to 34, with an OR of 6.0, 95% CI: 1.7-21.0. Hence, the identification of such a cut-off value requires larger studies, whereby the reliability of our result could also be confirmed.

6.4. Prediction of GDM at gestational weeks 32 to 34 by OGTT performed at gestational weeks 24 to 28

Although, GDM is manifested most often in the third trimester the negative result of an OGTT at gestational weeks 24 to 28 is of prognostic value as concerns the subsequent GDM. On the basis of this study, subsequent OGTT at gestational weeks 32 to 34 is recommended in the event of plasma glucose levels of ≤5.7 mmol/l and between 6.3 to 7.2 mmol/l at fasting and at 120 min, respectively, which is met merely by 36.6% of the population. Further testing seems to be omissible as subsequent GDM is very unlikely in the event of a plasma glucose level ≤6.2 mmol/l at 120 min. It seems that further testing may be omitted as subsequent GDM is very likely in the event of plasma glucose levels ≥5.7 and ≥7.3 mmol/l at fasting and at 120 min, respectively.

6.5. Summary of the thesis

1. Evaluation of HbA_{1c} in the early postpartum period seems to be a suitable method with which to detect antepartum undiagnosed GDM cases in the event of an increased neonatal birthweight (≥4000 g).
2. The proportion of women with an elevated postpartum HbA_{1c} level in the population of women with an increased neonatal birthweight (≥4000 g) was 9.8%, which corresponds to 24.8% of all GDM cases.
3. The best thresholds for the neonatal birthweight and maternal BMI with which to predict an elevated postpartum maternal HbA_{1c} level were 3695 g and 27.3 kg/m^2, but these do not seem to be worth clinical application.
4. Subsequent GDM is very unlikely in the event of a plasma glucose level of <6.3 mmol/l at 120 min of the OGTT at gestational weeks 24 to 28, thus, it seems that further testing may be omitted. We failed to identify such a fasting plasma glucose level (probably due to the relatively low case number).
5. Subsequent GDM is very likely in the event of plasma glucose levels of ≥5.7 mmol/l and ≥7.3 mmol/l at fasting and at 120 min, respectively, of the OGTT at gestational weeks 24 to 28, thus, dietary management should be considered instead of further testing.
6. Subsequent OGTT is recommended at gestational weeks 32 to 34 in the event of plasma glucose levels of <5.7 mmol/l and 6.3 to 7.2 mmol/l at fasting and at 120 min, respectively, which corresponds to merely 36.6% of the population.
7. We failed to identify cut-off fasting and postload glucose levels at gestational weeks ≤16 in a high-risk group, below which further OGTTs are unnecessary as the possibility of GDM is excluded. However, subsequent testing should be considered merely at gestational weeks 32 to 34 in the event of plasma glucose levels of <5.3 mmol/l and <6.8 mmol/l at fasting and at 120 min, respectively, for non-obese pregnant women.
8. Plasma glucose levels of ≥5.3 mmol/l and ≥6.8 mmol/l at fasting and at 120 min, respectively, at gestational weeks ≤16 in the event of obese pregnant women mean that further OGTTs could be unnecessary as the subsequent GDM is so strongly predicted.
9. Subsequent OGTT could be spared in 24.5% of the pregnant women at high risk of GDM by application of these cut-off values.
10. Obesity proved to be the only significant RF for GDM at gestational weeks 32 to 34, with an OR of 3.31, 95% CI: 1.32-8.29.

11. The positive predictive values of increased serum insulin levels at fasting (≥30 mU/l) and at 120 min postload (≥70 mU/l) at gestational weeks ≤16 for GDM at gestational weeks 24 to 28 in a high-risk group were 0.9 and 0.75, respectively. The negative predictive values of increased serum insulin levels at fasting and at 120-min postload at gestational weeks ≤16 for GDM at gestational weeks 24 to 28 in a high-risk group were 0.87 and 0.96, respectively.
12. The positive predictive values of increased serum insulin levels at fasting (≥30 mU/l) and at 120-min postload (≥70 mU/l) at gestational weeks ≤16 for GDM at gestational weeks 32 to 34 in a high-risk group were 0.92 and 0.87, respectively. The negative predictive values of increased serum insulin levels at fasting and at 120-min postload at gestational weeks ≤16 for GDM at gestational weeks 32 to 34 in a high-risk group were 0.53 and 0.73, respectively.

6.6. Recommended screening protocol for GDM, based on the results of these studies

According to the results of these studies, the following screening protocol for GDM was established, based on the risk assessment during the first prenatal or even preconceptional care:

6.6.1. Recommended screening protocol for women at average risk of GDM

The 75-g 2-hour OGTT should be performed at gestational weeks 24 to 28.
Interpretation of the result:
No need for further testing as subsequent GDM is very unlikely if there is:

- a plasma glucose level at 120 min <6.3 mmol/l

No need for further testing as subsequent GDM is very likely in the event of:

- a plasma glucose level at fasting ≥5.7 mmol/l
- a plasma glucose level at 120 min ≥7.3 mmol/l,

thus, dietary management should be introduced to prevent macrosomia and other complications.
Subsequent OGTT is recommended at gestational weeks 32 to 34 in the event of:

- plasma glucose levels of <5.7 mmol/l and 6.3 to 7.2 mmol/l at fasting and at 120 min, respectively

Subsequent testing is recommended, independently of gestational age and regardless of a previous negative result, in the event of:

- alterations in foetal development and amniotic fluid volume

Postpartum evaluation of HbA_{1c} is recommended in the event of a neonatal birthweight of ≥4000 g, regardless of the negative results of the previous OGTT during pregnancy.

6.6.2. Recommended screening protocol for women at high risk of GDM

The 75-g 2-hour OGTT with evaluation of the serum insulin should be performed at the preconceptional/first prenatal visit (at gestational weeks ≤16).

Interpretation of the result:

Pregnant women should be managed like those with a glucose intolerance, despite a negative OGTT result, in the event of:

- elevated serum insulin levels (≥30 mU/l and ≥70 mU/l at fasting and at 120 min, respectively)
- obese pregnant women with plasma glucose levels of ≥5.3 mmol/l and ≥6.8 mmol/l at fasting and at 120 min, respectively

Subsequent testing is recommended at gestational weeks 24 to 28 in the event of:

- a lack of insulin evaluation
- non-obese pregnant women with plasma glucose levels of ≥5.3 mmol/l and ≥6.8 mmol/l at fasting and at 120 min, respectively
- obese pregnant women with plasma glucose levels of <5.3 mmol/l and <6.8 mmol/l at fasting and at 120 min, respectively

Subsequent testing is recommended merely at gestational weeks 32 to 34 in the event of:

- normal serum insulin levels at fasting and at 120 min
- plasma glucose levels of <5.3 mmol/l and <6.8 mmol/l at fasting and at 120 min, respectively, for non-obese pregnant women

Subsequent testing is recommended independently of the gestational age and regardless of a previous negative result in the event of:

- alterations in foetal development and amniotic fluid volume

Postpartum evaluation of HbA_{1c} is recommended in the event of a neonatal birthweight of ≥4000 g, regardless of the negative results of the previous OGTT during pregnancy.

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8. ACKNOWLEDGEMENT

I wish to express my grateful thanks to my Ph.D. supervisor, Professor Attila Pál M.D., Ph.D., Director of the Department of Obstetrics and Gynaecology, for his continuous support, encouragement and professional guidance.

I am deeply indebted to Professor Emeritus László Kovács M.D., D.Sc., Director of the Reproductive Health Ph.D. Programme, former Director of the Department of Obstetrics and Gynaecology, for fully supporting my scientific activity.

I am especially obliged to Professor György Falkay D.Sc., Director of the Department of Pharmacodynamics, as my former supervisor in my research work.

I am gratefully obliged to Professor Emeritus Ian D Cooke M.D., DSc. (Department of Obstetrics and Gynaecology (Jessop Hospital for Women), University of Sheffield, UK), to Professor Gyula Tamás M.D., D.Sc. (First Department of Internal Medicine, Semmelweis University, Budapest, Hungary) and to László G Puskás Ph.D. for their valuable comments, advice and instructions during the preparation of the manuscripts and this thesis.

I wish to express my thanks to Krisztina Boda Ph.D. (Department of Medical Informatics, University of Szeged), to Tibor Nyári Ph.D. (Department of Medical Informatics, University of Szeged) and to Ádám Gy Tabák M.D. (First Department of Internal Medicine, Semmelweis University, Budapest, Hungary) for helpful discussions and for excellent technical assistance in the statistical analysis.

I am especially grateful to my family for their patience and support.

APPENDIX

I.