SCREENING OF GESTATIONAL DIABETES MELLITUS, AND THE OBSTETRICAL IMPORTANCE OF C-PEPTIDE LEVEL RECORDED DURING SCREENING

Summary of PhD thesis

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List of abbreviations

ACTH	adrenocorticotropic hormone
ADA	American Diabetes Association
BGGI	borderline gestational glucose intolerance
BMI	body mass index
CGR	C-peptide-to-glucose ratio
FCGR	fasting C-peptide-to-glucose ratio
2CGR	2-hour C-peptide-to-glucose ratio
GCT	glucose challenge test
GDM	gestational diabetes mellitus
GH	gestational hypertension
HCG	human chorionic gonadotrophin
HPL	human placental lactogen
IR	insulin resistance
NRDS	neonatal respiratory distress syndrome
NGT	normal glucose tolerance
OGTT	oral glucose tolerance test
PE	pre-eclampsia
PRL	prolactin
SD	standard deviation
SP 1	Schwangerschafts-protein 1, beta-1- glikoprotein
TNF	tumor necrosis factor
WHO	World Health Organization

1. Introduction, literature overwiev

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with first recognition during pregnancy. The definition applies irrespective of whether insulin or dietary only treatment is utilized and whether the condition persists after pregnancy. It does not exclude the possibility that unrecognized glucose intolerance may have antedated or begun concomitantly with the pregnancy. Most often this abnormal glucose tolerance returns to normal postpartum. Reclassification of maternal glycemic status should be performed at least 6 weeks after delivery. The prevalence of GDM ranges from 1 to 14 % of all pregnancies, however it depends on the diagnostic criteria used and the ethnic background of the population being tested. Earlier reports from Hungary reported that the frequency of GDM was between 1-3%. More recent reports from our country however detected higher frequencies up to 7.7%. The term GDM was introduced by *O' Sullivan* in 1961. The glucose intolerance of GDM is usually mild but perinatal mortality associated with this complication is 4 to 5 times increased compared to the general pregnant population. Macrosomia, intrauterine growth retriction, neonatal cardiomymiopathia, hypoglycaemia, jaundice, polycythemia, neonatal respiratory distress syndrome (NRDS) and hypocalcaemia may also effect newborns of GDM mothers. Offsprings of women with GDM are at increased risk of obesity, glucose intolerance, and diabetes in late adolescence and young adulthood. GDM is associated with an increased frequency of maternal hypertensive disorders and need for caesarean delivery. Moreover, GDM also considerably increases the woman's risk of developing manifest diabetes later in life. Thus, it is important to recognize and treat this disease. Obesity and other factors that promote insulin resistance appear to enhance the risk of type 2 diabetes after GDM, while markers of islet cell-directed autoimmunity are associated with an increase in the risk of type 1 diabetes.

The mechanisms involved in the development of this temporary diabetic state are still partly unknown. Among the possible explanations are reduced insulin secretion, increased

insulin degradation, increased secretion of hormones with anti-insulin effect (human placental lactogen [HPL], human chorionic gonadotrophin [HCG] prolactin [PRL] Schwangerschafts-protein 1, beta-1- glikoprotein [SP 1], estrogen, progesterone, cortisol and adrenocorticotropic hormone [ACTH]), reduced tissue sensitivity to insulin, or a combination of two or more these mechanisms. As glucose tolerance in pregnancy decreases in parallel with increasing levels of pregnancy-related hormones and of cortisol (until 37 week of gestation), it has been suggested that one or more of these hormones might be implicated in bringing about alterations in carbohydrate metabolism. Recently, the role of tumor necrosis factor (TNF) alfa and leptin has been suggested as they have numerous effects on carbohydrate metabolism. Pregnancy is a state of insulin resistance. In late pregnancy fasting serum insulin concentration is almost doubled compared to postpartum levels, both in normal pregnant and in gestational diabetic women. However, the insulin response to oral glucose or a mixed meal is significantly greater in normal pregnant women than in the gestational diabetic subjects. GDM women compared to normal pregnant controls have relative insulin-deficiency. An increased insulin demand of normal pregnancy is assured by the increase in the number and the size of islets of Langerhans.

If insulin production in unable to compensate for the insulin resistance (IR) of pregnancy, GDM will develop. Most GDM cases develop between the 24 and 28 weeks of gestation. This is the period hen in the absence other risk factors screening of GDM should be performed in all pregnant patients by a 75-gram OGTT (oral glucose tolerance test). Undiagnosed and untreated patients with GDM as well as their fetuses are at a higher risk of pregestional diabetes. Obstetric and perinatal outcomes in newborns of gestational diabetic women are related to metabolic control and fetal surveillance during pregnancy. If gestational diabetic subjects receive optimal care during gestation, the risk of maternal complications and perinatal morbidity is similar to those observed in normal pregnancies.

Since GDM has no signs or symptoms, it can only be recognized by screening. GDM is screened by a number of different methods. Screening for GDM is universally recommended despite the lack of consensus about the optimal screening method. The American Diabetes Association (ADA) recommends a two-step approach: All pregnant women should be screened for GDM between 24 to 28 weeks of gestation using a 50-gram 1-hour oral glucose challenge test (GCT) with a threshold for further testing of 140 mg/dl (7.8 mmol/l) or higher. The diagnosis of GDM is based on the 100-gram OGTT. Two or more of the venous plasma

glucose concentrations must be met or exceeded the values below for the diagnosis: fasting: 5.3 mmol/l, 1-h: 10.0 mmol/l, 2-h: 8.6 mmol/l, 3-h: 7.8 mml/l.

There is a controversy regarding the screening method for GDM. There are rational arguments both for a universal screening and for a selective screening within high risk groups. Selective screening for GDM is recommended according to the ADA. Low-risk pregnant women need no glucose testing by the ADA: Age < 25 years; weight normal before pregnancy; member of an ethnic group with a low prevalence of GDM; no know diabetes in first-degree relatives; no history of abnormal glucose tolerance; no history of poor obstetrics outcome. Using this criteria screening for GDM is not required in 10-36% of pregnant women, but up to 3% GDM remains undetected. For this reason all gravid women need to be screened for GDM. Risk screening for GDM: risk assessment should be undertaken at the first prenatal visit. Markers of high risk for GDM are shown in Table 1.

Table 1. Markers of high risk for GDM:

- Age (>35 years)
- obesity (BMI $\geq$ 30 kg/m^2)
- marked family history of diabetes or glycosuria
- personal history of GDM:
    - stillbirth
    - birth defect
    - macrosomia
    - weight gain
    - polyhydramnion
    - hypertension

Women with clinical characteristics consistent with a high risk of GDM should undergo glucose testing as soon as feasible. If they are found not to have GDM at that initial screening, they should be retested between 24 to 28 weeks of gestation.

Until 1995 the screening of GDM only included women with glucosuria or with a positive family history of diabetes in our hospital. At that time the incidence of GDM was only 1.11%. Between 1995 and 1997 general screening for GDM was initiated with a 40-gram standard breakfast test meal (1 roll of bread and 2 decilitres of milk). At that time the incidence of GDM increased to 2.07%. Since the incidence of GDM was much lower than the

valid figure for the rest of the country, general screening with the 75-gram OGTT was initiated in all pregnancies between 24 and 28 weeks of gestation was started in Szekszárd in 1997. GDM was diagnosed if the fasting value was at or above 7.0 mmol/l, or 2-h post challenge value was at or above 7.8 mmol/l (World Health Organization /WHO/).

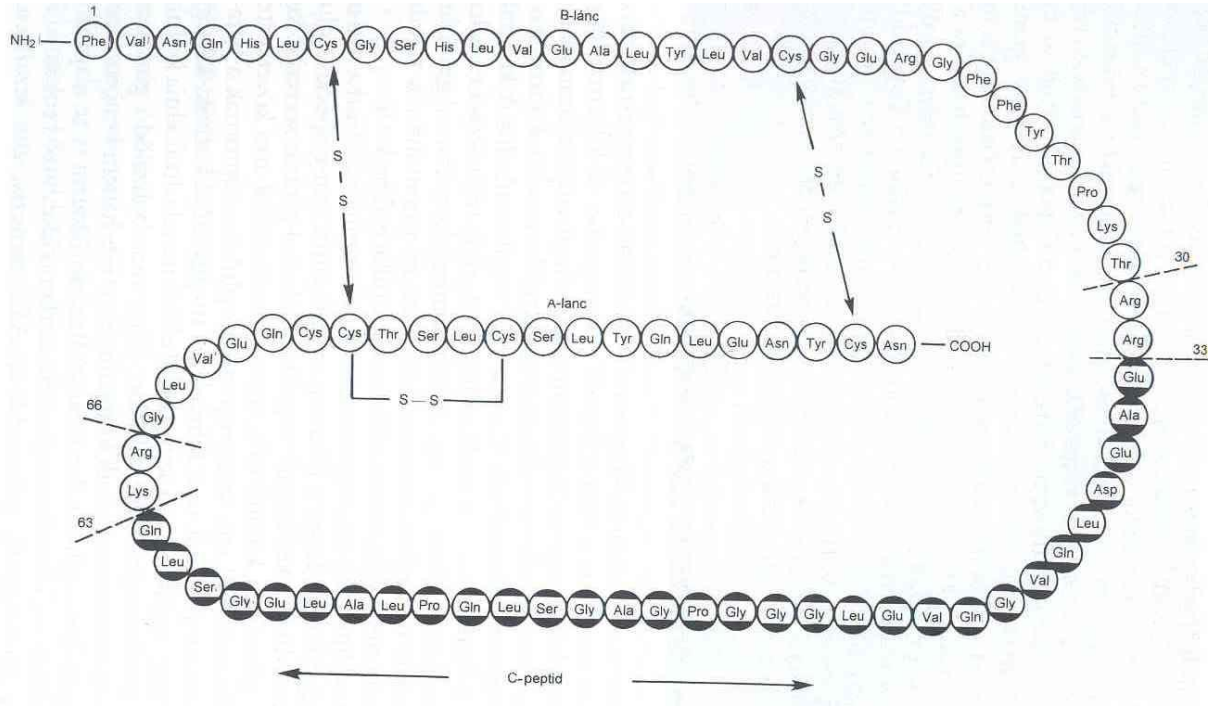
Parallel with the increasing levels of placental hormones till 37 weeks of pregnancy, the diabetic impact of pregnancy also increases. Consequently if glucose levels are around the upper limit of the normal range between 24 and 28 weeks of gestation these might further increase and cross the cut-off values in subsequent weeks. Due to the above if the OGTT result are normal, but the fasting glucose was between 6.0 and 7.0 mmol/l and/or a postload glucose between 6.8 and 7.8 mmol/l (glucose levels around the upper limit of the normal range) repeat testing was recommended within a month. After a pilot done on 136 women who were invited to a repeated screening 67 actually participated (49.3%) and GDM was diagnosed in 20 of the 67 (29.9) in 1999. The incidence of GDM increased from 3.9% without the repeat screening to 5.1%. In the light of these results we routinely recommended the repeat screening to women with these borderline values in the whole of Tolna County .

Hypertensive disorders in pregnancy are major causes of maternal, fetal and neonatal morbidity and mortality. They are associated with an elevated risk of seizures, stroke, hepatic and renal failure, coagulation disturbances, placental abruption, intrauterine growth restriction, fetal distress, premature delivery, and death. Approximately 6 to 9 % of pregnancies are complicated by gestational hypertension (GH), while pre-eclampsia (PE) occurs in 3 to 4% of pregnancies, usually in the second or third trimesters. It may also present up to six weeks post-partum.

The pathophysiology of hypertensive disorders in pregnancy is poorly understood but it is likely to be multifactorial. Several studies suggest that glucose intolerance and insulin resistance (IR) may play a role in the aetiology of these diseases, as the incidence of hypertensive disorders is twice as high in pregnancies of glucose intolerant women compared to women with normal glucose tolerance (NGT). The levels of C-peptide in the blood are measured instead of insulin levels and used as an indicator of IR. Insulin is initially synthesized in the form of proinsulin. In this form the A-and B-chains of active insulin are linked by a third polypeptide chain called the connecting peptide, or C-peptide, for short /Figure 1/. Equimolar amounts of C-peptide and insulin release from pancreatic beta cells to the portal circulation. IR and related hyperinsulinemia are associated with essential hypertension in non-pregnant individuals. In addition to the direct role in glucose metabolism,

insulin may additionally modify other physiological pathways that are indirectly or directly involved in sodium and water balance, and vascular resistance.

Figure 1. Human proinsulin



The prevalence of elevated blood pressure is doubled among overweight patients compared to normal-weight subjects. Furthermore, the observations of *Pollare et al.* suggest that the association between hypertension and IR is independent of obesity: relative IR was found not only in obese but also in lean hypertensive subjects compared to normotensive ones. Physiological changes during pregnancy lead to a decrease in insulin-sensitivity. As gestation advances, a progressive increase in insulin production to glucose can be found in the maternal pancreas. It has been shown that obesity plays a role in the development of IR in pregnancy. Patients with GDM are more insulin-resistant compared to control pregnant subjects with normal glucose tolerance. GH has been associated with hyperinsulinemia. No similar associations were reported regarding the relationship between PE and IR either in normoglycemic pregnancies or in GDM. PE is associated with a failure of trophoblast invasion to the spiral arteries of the placenta and it may lead to impaired uteroplacental perfusion and to the release of several vasoactive factors into the maternal circulation that finally causes endothelial dysfunction, vasoconstriction, and hypertension.

2. **Aims**

Based on the issues detailed in the introduction, our aims of were the following. The goal of first part of this study was to examine the incidence of gestational pathology in Tolna County, where 240 thousand people live and to show that the repeated screening for GDM is worthwhile. Until 2000 no data was available on the incidence of GDM in the whole county. The aim of this study was to determine the usefulness of a repeated screening for GDM during a given pregnancy in a population-based study in our country, too. The author also sought to clarify the role of certain risk factors (age, BMI, and number of pregnancies) in detail separately.

To our best knowledge, there are no population-based studies comparing IR values between women with GH and PE taking into account the potential effect of pre-pregnancy body mass index (BMI) on this association. In the second part of study we intended to investigate any possible correlation between IR and subsequent GH and PE in normoglycaemic and gestational diabetic pregnant women. Furthermore, we aimed to assess whether this correlation was independent of maternal weight.

3. Patients and methods

The first part of the study: It is a population-based screening program. The screening test (75-gram OGTT) was offered to all pregnant women without previously diagnosed diabetes in the city of Szekszárd since 1997. The program was extended in 1999 to cover the whole of Tolna County. Tolna County has a population of approximately 240,000. The 75-gram OGTT was performed according to WHO recommendation between 24 and 28 weeks of gestation.

Venous blood samples were collected following an overnight fast (≥ 8 hrs) and 2 hour after glucose ingestion. All glucose samples were measured by a glucose oxidase kit (Diagon Ltd, Hungary). According to WHO criteria, GDM was diagnosed if the fasting glucose was ≥ 7.0 mmol/l, or the 2-hour glucose ≥ 7.8 mmol/l. If the OGTT result was negative, but fasting glucose was between 6.0 and 7.0 mmol/l and/or a post-load glucose was between 6.8 and 7.8 mmol/l a repeat OGTT was scheduled within a month.

Data on age, pre-pregnancy anthropometric measures, and parity was collected by questionnaires sent to the district nurses who organized the care of all pregnant women in the county during 2000. Pre-pregnancy body mass index was calculated as pre-pregnancy weight in kilograms divided by height in meters squared.

Gestational age was determined on the basis of the woman's last normal menstrual period if it coincided within 1 week of the date determined by ultrasound done between 16 and 20 weeks of gestation, otherwise we used the ultrasound estimates.

The study design was reviewed and approved by the local Ethical and Research Committees.

Data are shown as mean ± standard deviation (SD) or percentages. Comparisons between groups were made using chi-square test for categorical and 2-sample t-tests for continuous variables. The threshold of statistical significance was set at $p<0.05$. Binomial proportions were used to estimate 95% confidence intervals around the observed frequencies.

The second part of the study: This population-based study was carried out in the Tolna County Balassa Janos Hospital between 1 August, 2001 and 1 March, 2007. The study design was reviewed and approved by the local Ethical and Research Committees, too. All women received written information on the aims and procedures related to the study from their attending obstetricians. During the study period that lasted for more than 5 years altogether 5,962 deliveries were registered in the hospital database. Of these deliveries 317 (5.3%) were preterm deliveries, 65 (1.1%) twin gestations, and 1,566 (26.6%) Caesarean deliveries. Of these women a total of 5,671 (95.1%) had OGTT results available and registered in the Tolna county hospital. The main reasons for the missing OGTTs were the following: women did not go for GDM screening, or they did not go to prenatal care, or only fasting blood glucose was measured, or 40-gram carbohydrate load was utilized.

The serum glucose levels during the diagnostic OGTTs were analyzed in 3 laboratories reflecting the dwelling-places of the participants. C-peptide determination (fasting and 2 h)

was however performed only in one of these laboratories, thus leading to the exclusion of 1979 pregnancies without C-peptide determination. Of the remaining 3,692 women further 738 were excluded due to delivery in another place (n=647), preterm delivery (<36 weeks of gestation, n=35), twin gestations (n=38), and due to prepregnancy or early pregnancy hypertension (n=18) leaving 2,954 records for the current analysis. (The twin pregnancies were excluded as these might lead to heightened IR owing to a larger placental mass). Serum glucose was analysed using a glucose oxidase kit (Diagon Ltd. Hungary), C-peptide was determined by a competitive radioimmunoassay (Biodata, Rome, Italy; coefficient of variation: 1.4%/1.0 ng/ml; sensitivity: <0.01 ng/ml of the C-peptide kit).

Blood pressure was measured after a 10-minute rest with the patient in a sitting position at the first obstetrical visit, and in the middle of gestation (between 18 and 22 weeks of gestation), then weekly after 36 weeks of gestation. Blood pressures were measured in duplicate, 5 minutes apart. Chronic hypertension was defined as a blood pressure of 140/90 mm Hg or higher on two occasions before 20 weeks of gestation, or persisting beyond 12 weeks postpartum. GH was defined as elevated systolic (≥140 mmHg) and/or diastolic (≥90 mmHg) blood pressure on at least two occasions 6 h apart after 20 weeks of gestation. PE was defined as GH in association with proteinuria (≥300 mg/24-hour period), in the absence of urinary tract infection.

The control group (control NGT) was composed of 2,583 normotensive subjects with normal OGTT results. The control group of the GDM (control GDM) was composed of 139 normotensive pregnant women with gestational diabetes.

GDM was diagnosed if the fasting serum glucose level was ≥7.0 mmol/l or the 2-hour postload serum glucose was ≥7.8 mml/l.

The C-peptide-to-glucose ratio (CGR) was calculated as the ratio of serum C-peptide (milimoles per liter -ng/mlx0,331=mmol/l-) to serum glucose (mmol/l). The ratio of serum C-peptide to serum glucose can refer to IR: higher values indicate IR, lower value indicate higher insulin sensitivity.

Pre-pregnancy BMI (pre-pregnancy weight in kilograms divided by height in meters squared) was calculated. Pre-pregnancy anthropometrical data was drawn from the records of the district doctors collected at the first obstetrical visit. Maternal weight gain was defined as the difference between the final and initial maternal weight. The final maternal weight was measured in the delivery room.

Lower BMI subgroup: Since the BMI values were significantly different between the hypertensive groups (PE NGT=25.9 kg/m^2 GH NGT=27.4 kg/m^2 PE GDM=28.3 kg/m^2 GH GDM=31.8 kg/m^2) and the respective control groups (GDM 23.1 kg/m^2, without GDM 25.4 kg/m^2), a lower BMI subgroup was formed within the PE and GH groups with and without GDM. Starting with the women with the lightest weight of GH and PE groups women were added individually to the lower BMI (l.BMI) group until the mean BMI value of the respective control group was reached (non-GDM women: PE l.BMI NGT=22.9 kg/m^2, GH l.BMI NGT=23.6 kg/m^2 versus control NGT=23.1 kg/m^2, GDM women: PE l. BMI GDM=25.9 kg/m^2 GH l. BMI GDM=25.8 kg/m^2 versus control GDM=25.4 kg/m^2). With the use of these l. BMI groups, we could investigate whether the IR differences between in different hypertentensive and normotensive groups were confounded by higher BMI.

Data were shown as mean ± standard deviation (SD). Statistical analyses were performed using Chi-square tests for categorical and one-way analysis of variance (ANOVA) for continuous variables. Statistical significance was inferred at a two-tailed $P < 0.05$.

4. Results

In the first part of the study the results were the following: 2,281 babies out of the 2,260 deliveries were born in our county during 2000. Detailed information was collected on standardized questionnaires by district nurses on 2,138 (94.6%) of there pregnancies. Further 125 pregnancies were excluded where the OGTT data was incomplete leaving a final sample of 2,013 for further analysis (Table 2). Of these GDM cases 167 (8.3%, 95% confidence interval [CI]: 7.1-9.6%) were diagnosed during the first OGTT (24-28 weeks of gestation). Intermediate (non-diagnostic) results were obtained in 216 women (10.7% 95%CI: 9.4-12.2%) and thus they were invited for a further testing within a month.

Of the 216 invited, only 143 (66.2%) participated in the repeat examination. Among the 143 participants 20 (14.0% 95%CI: 5.3-27.9%) GDM cases were diagnosed during the repeat screening. After all out of 2,013 women, 187 (9.3%) were diagnosed with GDM. If all 216 women had gone to the repetition, then probably 30 women would have had GDM instead of

20. Thus the prevalence of GDM would be 9.8% instead of 9.3%.By the extrapolation of these binomial proportions to the 216 invited women, we would expect 30 (95%CI 11-60) more GDM cases to be diagnosed.

Table 2. 75-gram OGTT has not been used (n=125)

Serum glucose determination did not happen	n	%
Women did not go to screening test for GDM	62	49.6 %
Patients did not go to prenatal care	27	21.6 %
Fasting serum glucose has been used only	**n**	**%**
Pregnant subjects vomited glucose solution	10	8.0 %
Fasting blood glucose has been planned only	9	7.2 %
Women refused to drink the glucose solution	5	4.0 %
A loading test containing 40-gram carbohydrate has been used	12	9.6 %

The characteristics of 187 GDM women compared to 1826 controls are listed in Table 3: GDM women were significantly older, had a higher BMI and increased fasting and postload glucose during the OGTT.

Although a linear increasing trend of GDM risk was obvious in all 3 investigated parameters (age, BMI, number of pregnancies) according to the χ^2-tests, surprisingly the highest point estimates were found not in the groups within the highest strata: GDM risk was

highest in 30-39 yrs old women (14.4%), in women with a BMI of 25-29.9 kg/m^2 (13.5%), and during 3rd pregnancies (14.2%). It seems that the risk does not increase any further with an increasing level of the risk factor beyond the previously described groups.

Table 3: Characteristics of screened women by GDM status

	Norm. OGTT n=1826	GDM n=187	all mother n=2013
Age (yars)	26.3± 5.2	28.6± 5.4*	26.5±5.0
Pregravid BMI (kg/m²)	23.1±4.6	24.7±4.7*	23.4±4.5
Number of pregnancies	2.3±2.0	2.6±2.2	2.4±1.7
Parity	2.0±1.3	2.2±1.2	2.0±1.2
Fasting blood glucose (mmol/l)	4.3±1.2	4.9±0.8*	4.4±1.3
Postload blood glucose (mmol/l)	5.3±1.5	8.8±1.3*	5.4±1.4

* p<0.01 GDM vs. normal OGTT Mean±SD

In the second part of study period, 2,954 expectant women were included in the study, 183 (6.2%) developed GH and 49 (1.7%) PE. GDM was diagnosed in 6.0 % of the participants (176/2,954). The incidence of GH among GDM women was 15.9% (28/176) and of PE was 5.1% (9/176) compared to 5.6% (155/2,778) and 1.5% (40/2778) among normoglycemic pregnant women.

Gestational weight gain was also significantly larger among women who developed PE than in control women either with GDM (p<0.01) or without GDM (P<0.02). No differences were found between the control and the GH groups in gestational weight gain, except that weight gain was significantly different between the GH l. BMI group and the respective control group (P<0.05). The different hypertensive groups were similar to their respective control groups (with and without GDM) in respect to the mother's age at delivery.

There were no significant differences in serum glucose levels between controls (with or without GDM) and any of the hypertensive groups. The fasting and 2-hour C-peptide concentrations among subjects in PE (with or without GDM) were similar to those of the control groups [normotensive group with NGT (n=2,583) or with GDM (n=139)]. The fasting and 2-hour C-peptide levels were significantly higher ($p<0.05$) among women who developed GH (with or without GDM) than among the respective normotensive control groups (with or without GDM), except the difference between the GH l. BMI GDM and control GDM group.

The fasting CGR (FCGR) and 2-hour CGR (2CGR) values among subjects in the PE (with or without GDM) were similar to those in the respective normotensive groups (with or without GDM). The FCGR and 2CGR values were significantly higher in all women with GH irrespective of BMI, compared to the normotensive group. (Except the difference between the 2-hour GH GDM l. BMI and 2-hour control GDM). We can ascertain that there is significantly higher CGR in GH with and without GDM. It is independent of maternal weight, too.

5. Discussion and conclusion

The first part of this study shows that the incidence of GDM is higher than it had been thought in Hungary (9.3%, without repetition: 8.3%). Additional two studies involving Hungarian pregnancies in large populations verified this result in 2006 and in 2008. Three research studies proved strongly that pregnant Caucasian Hungarian patients are at high risk for GDM (8.2-9.3%).

Randomized clinical trials support the importance of the treatment of even the mildest forms of gestational glucose intolerance. Although treatment of this borderline gestational glucose intolerance (an abnormal glucose challenge test followed by a normal 100 gram OGTT) did not significantly reduce the frequency of stillbirth or perinatal death and neonatal complications, it did however reduce the risk of fetal overgrowth, and in some studies also shoulder dystocia, caesarean delivery, and hypertensive disorders among the mothers. Although there are no studies demonstrating that treatment would decrease the risk of complications in women with GDM diagnosed by a repeated test, based on the randomized

trials in borderline gestational glucose intolerance we hypothesize that pregnancy outcomes might be improved by the repeated screening.

The results of the first part of this study demonstrate that repeated testing increases the incidence of GDM slightly, thus the outcome of these pregnancies might be improved by recognizing these additional cases of GDM. It is also important to find ways to improve the uptake of the screening test if the pilot is to be continued. The author found a higher uptake of the repeated screening in the city of Szekszárd (90.4%) suggesting that local factors, probably related to the characteristics of the care provider are related to the success of the program. In conclusion, the author reports that a substantial number of gestational diabetes cases may be diagnosed if the screening OGTT is repeated 4 weeks after the recommended 24-28 weeks of gestation. Further studies are required to determine whether these additional GDM cases have an increased maternal or fetal risk.

Previously several risk factors for GDM were described such as older age, significant obesity, glycosuria, positive family history of diabetes, previous maternal history of a macrosomic infant, unexplained stillbirth, and previous GDM. The results of this study confirm the role of maternal age, pre-pregnancy BMI, and parity as risk factors for GDM development. Furthermore the current study shows that the risk of GDM did not increase further over 40 yrs of maternal age, over a BMI of 30 kg/m^2, and even decreases in women with more than 3 pregnancies compared to women with 3 pregnancies. The finding that GDM risk is decreased in women with more than 3 pregnancies might suggest a self-selection of healthy women in this population. Furthermore we found that the risk of GDM levels off above the age of 40 yrs and a BMI of 30 kg/m^2 that also supports a healthy selection hypothesis.

The second part of the present study shows that the level of IR is not increased in PE (with NGT or with GDM) compared to the respective normotensive women. FCGR and 2CGR were significantly higher among women with GH compared to normotensive women. This difference exists even in the lower BMI subgroups, suggesting that other factors than obesity might also be involved in the development of IR among GH women.

IR is defined as an impaired (poor) glucose response to either exogenous or endogenous insulin. IR has been quantified by a number of different methods. Among these measures the euglycaemic-insulin-clamp-derived and the minimal-model-based determinations are not suitable for large-scale studies due to their high costs and labour intensity, while estimations using fasting insulin (or C-peptide) levels are much cheaper and easier to perform. It is

generally accepted that (very) high plasma insulin levels in the setting of normal glucose levels are likely to reflect IR. C-peptide supposed to be a superior measure to insulin in the estimation of IR, since insulin levels are significantly affected by its clearance. In the present study, IR was estimated using the CGR method. The CGR can be easily calculated in daily clinical practice to establish IR.

IR develops normally during late pregnancy even in women with normotension and NGT. All previous reports found hyperinsulinaemia in GH compared to normotenisve pregnancies. This observation was also confirmed in the present study, while the relationship between PE and IR is equivocal. This study, similarly to some previous reports, we found no relationship between PE and IR. PE and GH probably have different aetiologies with different pathophysiological mechanisms, as supported by previous data and by our current results.

It is also hypothesized that obesity contributes to the development of IR in hypertensive disorders of pregnancy. Furthermore, it was suggested that the incidence of PE and GH rises sharply as BMI increases. A link between IR and later development of hypertensive disorders in pregnancy is also suggested by an association with increased BMI and excessive weight gain in previous studies. Except from one study in Japan, there are no reports investigating the association between IR and hypertensive disorders in pregnancy after taking into account differences the obesity.

Our report suggests that the association between IR and GH is independent of obesity. We suspect that the GCR might improve the prediction of GH, and thus high risk women might be screened at the time of the 75-gram OGTT (24-28 weeks of gestation). These women have to attend prenatal care. Unfortunately, our results suggest that the measurement of IR does not predict PE. Some data suggest that uterine artery blood flow velocity waveforms analysis could improve the prediction of this disease.

6. Summary of novel findings

1. This study shows that the incidence of GDM (8.2-9.3%) is higher than it has been thought in Hungary.

2. The author recommends repeated screening for GDM within a month if the glucose levels are around the upper limit of the normal range. The results of this study demonstrate that repeated testing increases the incidence of GDM slightly.
3. Efforts should be made to encourage pregnant women to take up the repeated test. District doctors in Szekszárd and its immediate surroundings reached a better uptake of the repeat screening: 103 (90.4%) of 114 women with borderline values actually participated. The success rate was much lower in other areas: 40 (39.2%) of 102 women participated.
4. We found that the prevalence of GDM is the largest in the BMI category of 25.0-29.9 kg/m^2, between 30-39 years of age, and at the third deliveries in detail separately.
5. The data of the present study provide indirect support for the hypothesis that higher CGR precedes GH, and IR may be an important player in the aetiology of vascular dysfunction in GH both in normal glucose tolerance and in GDM. We suspect that the GCR might improve the prediction of GH and thus high risk women might be screened at the time of the 75-gram OGTT (24-28 weeks of gestation). These women have to attend prenatal care.
6. On the other hand, this study does not support the hypothesis that IR is involved patohpysiology of pre-eclampsia, as no difference in IR was found in IR between the PE and the control groups either in normoglycemic or in gestational diabetic pregnant women. Our results suggest that the measurement of IR does not predict PE. Some data suggest that uterine artery blood flow velocity waveforms analysis could improve the prediction of this disease. By this means we would take care of the gravids with PE before the signs appear.
7. Furthermore, these relationships were independent of maternal obesity. Our observations confirm again that GH and PE are heterogeneous disorders.

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8. List of publications, presentations and posters

The thesis is based on the following publications:

Original articles:

1 **Dr. Kun A**: The incidence of gestational diabetes mellitus in Tolna county during 2000 Diabetologia Hungarica 2006 14 (3) 235-240

2 **A. Kun MD**, J. Tornóczky MD: Is there a relationship between insulin resistance and pre-eclampsia? International Procedings of the 8th World Congress of Perinatal Medicine. Monduzzi Editore, Bologna, 2007, pp 523-527.

3 **A. Kun MD:** Estimated incidence of gestational diabetes mellitus in Hungary Diabetes Research and Clinical Practice (Letter to the Editor) 2009 (3) 83.

IF: 2.160

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