CLINICAL STUDY

Overweight is associated with impaired β-cell function during pregnancy: a longitudinal study of 553 normal pregnancies

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Abstract

Objective: To monitor β-cell function and insulin sensitivity longitudinally in a large cohort of pregnant women to elucidate mechanisms that influence glycemic control in pregnancy.

Design and methods: Five hundred and fifty-three pregnant Scandinavian women underwent 75 g oral glucose tolerance test (OGTT) at weeks 14–16 and 30–32. Insulin sensitivity (Matsuda index) and β-cell function (ratio of AUCinsulin to AUCglucose, AUCins/glc) were calculated from 520 complete tests, and subsequently β-cell function was adjusted for insulin sensitivity, rendering an oral disposition index (DIo).

Results: Eleven women (2.1%) had gestational diabetes mellitus (GDM1) at weeks 14–16, and 49 (9.4%) at weeks 30–32 (GDM2), which is higher than that previously reported in this region. In the subdivision of OGTT, more overweight (body mass index ≥ 25) was found in glucose-intolerant groups (glucose-tolerant women (normal glucose tolerance, NGT) 38 versus GDM2 women 58 and GDM1 women 82%, P < 0.005). In early pregnancy, insulin sensitivity was lowest in GDM1, intermediate in GDM2, and highest in NGT. In late pregnancy, insulin sensitivity decreased in all groups, most in gestational diabetes. β-cell function demonstrated minor shifts during pregnancy, but when adjusted for decreasing insulin sensitivity, DIo levels fell by 40% (P < 0.001). DIo was significantly attenuated relative to glucose intolerance (GDM1 25% and GDM2 53%) during pregnancy. In overweight women, DIo levels were lower throughout pregnancy (P < 0.001 versus normal weight women), this reduction was significant (P < 0.01) in both NGT (21–25%) and GDM2 subjects (26–49%).

Conclusion: β-cell function adjusted for insulin sensitivity (DIo) deteriorated during pregnancy in both glucose-tolerant and glucose-intolerant women. The failure to compensate the decrease in insulin sensitivity was accentuated in overweight women.

Introduction

During gestation, there is a gradual decrease in insulin sensitivity (1). A variety of endocrine factors including estrogens, progesterone, human placental lactogen, cortisol, and tumor necrosis factor-α seem to impair the effect of insulin (2, 3).

In most pregnant women, some increase in post-prandial glucose levels is seen, and in some, mild increases in fasting plasma glucose (FPG) levels are also seen (4). In normal circumstances, euglycemia is maintained during pregnancy due to compensatory hyperinsulinemia. The inverse relationship between the acute insulin response and insulin sensitivity during i.v. glucose tolerance tests is known as the disposition index (DI), and is constant for an individual at a given glucose tolerance level (5, 6).

The increasing prevalence of obesity in fertile age (7) contributes to lower insulin sensitivity, and could stress β-cell function during pregnancy resulting in an increased risk of gestational diabetes mellitus (GDM). Gestational diabetes identifies women at high risk of developing type 2 diabetes, and can provide a model of early events in the development of the disease (8). The physiological increase in insulin resistance (IR) in pregnancy can unmask a previously unknown β-cell defect in some individuals (9). Women with GDM have been reported to have lower DI during and after pregnancy compared to normoglycemic individuals (9). In addition, GDM is associated with increased risk of maternal and fetal morbidity and with complications on short- and long-term perspective (10, 11). Milder dysglycemia (nonGDM) results in intermediate rate of complications, indicating no threshold between the level of glycemia and adverse pregnancy outcome (12).

For assessment of insulin sensitivity, the euglycemic clamp is the reference method (13). However, in larger studies, simplified tests are used such as the oral glucose tolerance test (OGTT). In recent years, a number of
indices of insulin sensitivity and β-cell function based on OGTT data have been developed (reviewed in (14)). Validation of such insulin sensitivity indices with clamp studies in pregnancy has been performed (15), and to our knowledge, similar evaluations for β-cell function in pregnancy have not been reported.

There are few prospective studies of β-cell function compared to studies of insulin sensitivity in pregnancy. Decreases in β-cell function are to some degree masked by decreasing insulin sensitivity during pregnancy (16). The DI was developed to adjust for compensatory high insulin responses to a decreased insulin sensitivity based on data obtained from i.v. glucose tolerance tests (6). Recently, the hyperbolic relationship of β-cell function and insulin sensitivity has also been demonstrated with data obtained from OGTT results in nonpregnant individuals (17, 18), and this relationship has not been tested in pregnancy. In pregnant women in late gestation (weeks 24–28), β-cell function adjusted for insulin sensitivity deteriorated markedly when impaired glucose tolerance (IGT) and GDM individuals were compared with normal glucose tolerance (NGT) individuals, and most so in GDM (19). A recent prospective study in pregnant women of normal weight found falling insulin sensitivity and failing β-cell function in overt GDM (4). It also plotted the change during pregnancy in β-cell function (ratio of AUC_{insulin} to AUC_{glucose}, AUC_{ins/gluc}) against insulin sensitivity, and could demonstrate a significant defect in compensation for IR in subjects with early onset of GDM, while late-onset GDM subjects did not differ in adjusted β-cell function when compared to NGT subjects.

The STORK study investigated healthy pregnant Scandinavian women prospectively to evaluate the effects of metabolic markers and anthropometry on pregnancy outcome.

Previous analyses in this cohort indicate that subgroups of overweight women have dissimilar risks of delivering macrosomic newborns depending on the change in fasting glucose throughout pregnancy (20). Normal pregnant women in the upper body mass index (BMI) quartile (BMI above 27 kg/m²) with the highest increase in FPG from early to late pregnancy had a 4.5-fold increased risk of delivering a macrosomic infant irrespective of insulin levels. There were no effects of gestational weight gain or macronutrient intake on the risk of macrosomia, nor was there a significant interaction between physical inactivity and BMI (21). The absence of effect on macrosomia of insulin and homeostasis model for assessment of insulin resistance (HOMA-IR) was contrasted by a robust effect of fasting glucose which could indicate that IR is not a major mechanism behind the association between dysglycemia and newborn macrosomia (20). Thus, significant increases in FPG in a subgroup of overweight women with large newborns could possibly result from a subtle β-cell dysfunction during pregnancy.

The aim of the current study was therefore to evaluate β-cell function and insulin sensitivity prospectively in a longitudinal cohort of pregnant women with a view of elucidating possible differences between early and late pregnancies in the overall metabolic state using surrogate dynamic indices derived from the OGTT. Furthermore, we wished to explore the impact of adjusting β-cell function estimates for the decreases in insulin sensitivity seen in pregnancy in this cohort.

Materials and methods

This prospective study was based on a cohort of 553 women (Table 1) described in detail elsewhere (21). Women with Scandinavian heritage were invited to participate. Exclusion criteria were multiple pregnancies, known diabetes, fetal malformation, or other severe maternal illnesses.

The women came for four antenatal visits during pregnancy. At gestational weeks 14–16 and 30–32, 75 g OGTTs were performed. In addition, at weeks 22–24 and 36–38 anthropometry was done. Five hundred and twenty participants had OGTT results permitting categorization into glucose tolerance groups. Thirty-three participants had incomplete OGTT data due to preterm birth, hospitalization, difficult venous access, or nausea during the first OGTT. The participants were stratified as glucose tolerant (NGT) or GDM according to the 2-h OGTT results (< 7.8 mmol/l (22)). Subjects with 2-h glucose levels > 7.8 mmol/l received dietary and lifestyle advice, and none of the GDM subjects required anti-diabetic medication during the study.

Independent variables

Glucose was measured immediately in EDTA blood by Accu-Chek glucose test strips and glucometer (Roche Diagnostics). Samples were collected in 7 ml Vaccutainer tubes, and centrifuged without delay at room temperature at 3000 g for 10 min. Serum was aliquoted immediately and stored at −80 °C until analyzed. Insulin samples were assayed in duplicate (RIA, DPC, Los Angeles, CA, USA), and the intra- and inter-assay coefficient of variation values were 4.9 and 5.4% respectively. Height was self-reported and weight was measured on a digital scale.

Table 1 Cohort characteristics.

<table>
<thead>
<tr>
<th>n = 553</th>
<th>Mean ± s.d.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>31.2 ± 4</td>
<td>19–42</td>
</tr>
<tr>
<td>Maternal weight (kg, weeks 14–16)</td>
<td>70.8 ± 12.1</td>
<td>44.6–123.1</td>
</tr>
<tr>
<td>BMI (weeks 14–16)</td>
<td>24.9 ± 4.1</td>
<td>17.5–44.0</td>
</tr>
<tr>
<td>Weight gain during pregnancy (kg)*</td>
<td>10.6 ± 3.8</td>
<td>−1.2–29.4</td>
</tr>
<tr>
<td>Gestational age at birth (weeks)</td>
<td>40.0 ± 1.8</td>
<td>28.4–43.1</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3619 ± 570</td>
<td>1275–5420</td>
</tr>
</tbody>
</table>

*Change in weight from early (weeks 14–16) to late (weeks 34–36) visits.
Outcome variables

Glucose and insulin levels, fasting or during the OGTT, were used for computation. We calculated AUC by the trapezoidal rule for glucose and insulin during OGTT, and β-cell function was given by the ratio of AUCinsulin to AUCglucose (AUCins/glc). The Matsuda index (ISogtt) was given by the formula \( \frac{10^000}{(FPG \times FPI \times (G \times I))} \), where FPG is fasting plasma glucose, FPI is fasting plasma insulin, and G and I represent the mean of glucose or insulin levels during OGTT, was calculated as an estimate of insulin sensitivity (23).

We found a hyperbolic relationship after plotting the ratio of AUC insulin to AUCglucose (AUCins/glc) against the ISogtt index, and subsequently calculated an oral (DIo = AUCins/glc × ISogtt) to adjust β-cell function for the effect of decreasing insulin sensitivity (17).

Statistical methods

FPG values had a normal distribution, and differences and changes were analyzed by paired or independent sample t-tests as appropriate. FPI values at all the four visits were skewed. Differences and changes in FPI and indices of β-cell function, insulin sensitivity, and IR were analyzed by nonparametric tests. Wilcoxon signed-rank test for paired samples was used to analyze change, and Kruskal–Wallis test and Mann–Whitney tests were used to compare groups, with Bonferroni corrections when appropriate. The distribution of overweight (BMI > 25 kg/m²) relative to glucose tolerance category was tested with the \( \chi^2 \) test. All analyses were done by SPSS version 15.0 (Chicago, IL, USA).

Ethics

The study was approved by the Regional Ethics Committee and performed according to the Declaration of Helsinki, and written informed consent was obtained from the participants.

Results

Table 1 shows the cohort characteristics. Only 4% of the participants were smokers, and 53% were primipara. Eleven participants (1.9%) had early-onset GDM (GDM1) at weeks 14–16 and 49 (9.4%) had GDM at weeks 30–32 (GDM2). None of the GDM cases had elevated fasting glucose (> 6.1 mmol/l).

Mean BMI at gestational weeks 14–16 was 24.9 kg/m², and mean change in body weight was 10.6 kg from weeks 14–16 to 34–36. However, women with GDM1 had significantly higher BMI than NGT subjects (28.3 vs 24.1 kg/m², \( P < 0.05 \)); only two women with GDM1 had BMI below 25 kg/m² at weeks 14–16. A subdivision of OGTT groups by BMI demonstrated a preponderance of overweight (BMI > 25) in glucose-intolerant states (NGT 38%, GDM2 58%, and GDM1 82% respectively, \( \chi^2 \) test, \( P < 0.005 \)).

Glucose and insulin levels during OGTT

Glucose and insulin levels demonstrated an upward shift from early to late pregnancy (Fig. 1) in normal weight women and more so in overweight women. However, serum insulin levels demonstrated great inter-individual variability in both groups.

![Figure 1: Glucose (upper panels) and insulin (lower panels) levels during OGTT for normal weight (BMI ≤ 25) and overweight women (BMI > 25). Each boxplot cluster depicts tests at weeks 14–16 (left) or 30–32 (right). * or □ are outliers, some s-insulin extremes are removed.](www.eje-online.org)
Insulin sensitivity indices

Changes in ISogtt from early to late pregnancy
Glucose-tolerant women had significant changes in ISogtt during pregnancy (P<0.0001). In women with GDM1, ISogtt tended to decrease (P<0.08). Women with GDM2 demonstrated significant (P<0.0001) reductions in ISogtt (Table 2).

Changes in ISogtt by glucose tolerance categories
ISogtt fell during pregnancy in NGT subjects. A greater relative reduction was seen in GDM2 subjects compared to NGT subjects, and the reduction was largest in GDM1 subjects. The difference in insulin sensitivity between GDM1 and GDM2 was most pronounced at weeks 14–16 (30%, P<0.01; Table 2).

Changes in ISogtt by BMI categories
In individuals with BMI <25, an effect of GDM2 was only found at weeks 30–32 when ISogtt was 35% lower (P<0.002) compared to glucose-tolerant individuals (Table 2).

In overweight women with GDM1 and GDM2, ISogtt decreased by ~40–60% in both early and late pregnancies compared to NGT women (P<0.003). ISogtt at weeks 14–16 was 33% lower (P<0.04) in overweight GDM1 subjects when compared to GDM2 subjects in the same BMI category.

β-cell function indices (AUCins/glc)

Changes in AUCins/glc from early to late pregnancy
Glucose-tolerant women had a significant increase in AUCins/glc during pregnancy (P<0.0001), but there was no significant change in GDM1 subjects. GDM2 subjects also demonstrated significant increases in β-cell function (AUCins/glc, P<0.0001; Table 2).

Changes in AUCins/glc by glucose tolerance categories
The levels of AUCins/glc were similar in all glucose tolerance categories in both early and late pregnancies (Table 2). The change in AUCins/glc levels during pregnancy between the categories was only a trend (P<0.07).

Changes in AUCins/glc by BMI categories
In overweight women with NGT, AUCins/glc at weeks 14–16 and 30–32 decreased by 12 and 18% respectively (both P<0.0003, Table 2) compared to NGT women of normal weight.

β-cell function adjusted for insulin sensitivity level (DLo)

The changes in β-cell function during pregnancy relative to insulin sensitivity are shown in Fig. 2. The plots show the expected hyperbolic relationship between estimates of insulin secretion and insulin sensitivity. In both early and late pregnancies, ISogtt was inversely correlated with AUCins/glc (r = −0.69 and r = −0.73 respectively, both P<0.0001).

The median DLo in early pregnancy was highest in NGT (137), followed by GDM2 (96) and then GDM1 (74), and this shift (Table 3, overall P<0.0001) supports the idea of a decline in β-cell function across these groups.

Change in adjusted β-cell function (DLo) from early to late pregnancy
The median DLo decreased by 28% from weeks 14–16 to 30–32 (P<0.0001). The reduction of adjusted β-cell function during pregnancy was 27% in NGT participants, P<0.0001. The impaired β-cell function was accentuated in the GDM2 group, where DLo fell by 35%, P<0.0001. The lowest DLo levels were found in GDM1 women, but these levels tended to decline only during pregnancy (18%, P<0.1; Table 3).

Table 2 Insulin sensitivity and β-cell function, median (25 and 75th quartiles) by glucose tolerance and body mass index (BMI) categories.

<table>
<thead>
<tr>
<th>Gestational weeks</th>
<th>Insulin sensitivity (ISogtt)</th>
<th>β-cell function (AUCins/glc)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14–16</td>
<td>30–32</td>
</tr>
<tr>
<td>NGT (n=460)</td>
<td>3.32 (2.37; 4.71)</td>
<td>41.3 (32.3; 55.9)</td>
</tr>
<tr>
<td>GDM1 (n=11)</td>
<td>1.45 (0.67; 1.66)*</td>
<td>55.7 (36.2; 99.4)</td>
</tr>
<tr>
<td>GDM2 (n=49)</td>
<td>2.09 (1.46; 3.17)*</td>
<td>43.3 (36.9; 55.6)</td>
</tr>
<tr>
<td>BMI ≤25 NGT (n=193)</td>
<td>3.8 (2.8; 5.2)‡</td>
<td>39.2 (30.4; 49.6)‡</td>
</tr>
<tr>
<td>GDM1§</td>
<td>3.1 (2.0; 4.2)‡</td>
<td>42.3 (32.4; 54.7)</td>
</tr>
<tr>
<td>GDM2 (n=20)</td>
<td>3.1 (2.0; 4.2)‡</td>
<td>55.0 (39.4; 81.9)</td>
</tr>
<tr>
<td>BMI &gt;25 NGT (n=267)</td>
<td>2.6 (1.9; 3.7)</td>
<td>47.4 (35.3; 64.6)</td>
</tr>
<tr>
<td>GDM1 (n=9)</td>
<td>1.2 (0.7; 1.7)*</td>
<td>75.6 (44.6; 113.5)</td>
</tr>
<tr>
<td>GDM2 (n=28)</td>
<td>1.8 (1.3; 2.4)*</td>
<td>45.5 (37.5; 56.5)</td>
</tr>
</tbody>
</table>

*P<0.05 for difference versus NGT, †P<0.05 for difference versus GDM2, ‡P<0.01 for difference between BMI categories. §BMI<25, n=2, data not computed.

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DIo changes between glucose tolerance groups

At weeks 14–16, DIo in GDM1 and GDM2 subjects was 46 and 30% lower respectively compared to NGT subjects (both \( P < 0.0003 \) (Table 3). Similarly, when GDM2 participants were categorized by BMI, overweight subjects had 33% lower DIo at both weeks 14–16 and 30–32 (both \( P < 0.0001 \)).

**Discussion**

In this prospective study, DIo estimates demonstrated definite decreases in \( \beta \)-cell function in all glucose tolerance categories, among overweight women as well as among normal weight women. AUC_{ins/gluc} increased during pregnancy, but did not reveal significant changes between glucose tolerance groups. We found the expected decline in dynamic insulin sensitivity during pregnancy, and these changes were accentuated with decreasing glucose tolerance. In overweight subjects, \( \beta \)-cell function did not differ markedly when categorized by glucose tolerance contrary to the deterioration of insulin sensitivity found in overweight subjects when categorized by glucose tolerance. In our cohort, approximately one-fifth of GDM subjects had an early onset of dysglycemia (weeks 14–16) and the rate of GDM was higher than that reported in the Medical Birth Registry (24). These data indicate that in low-risk populations, GDM may be underestimated.

Our cohort is well characterized and had good protocol adherence, consisting of a heterogeneous well-educated urban population of pregnant Caucasian women. The participants had higher pre-gestational weight than those who declined participation (67.2 vs 64.5 kg). Furthermore, those who did or did not participate were similar with respect to height, age, parity, smoking habits, marital status, education level, or employment outside home (21).

The OGTT method is considered less physiologic than meal tests due to the large glucose load, and carbohydrate intake previous to overnight fast could also influence the test results. However, the test is simple to perform and is still recommended for diagnostic use in pregnancy (22), in spite of the less-than-perfect reproducibility (25).

Indices of \( \beta \)-cell function and insulin sensitivity based on peripheral insulin levels are estimates, and could be influenced by altered hepatic extraction. Also, high variability of insulin responses might influence the reliability of computed indices, as is known from the HOMA indices (26). Validation of indices of insulin sensitivity in pregnancy has been done in women with NGT (\( n = 10 \)) and GDM (\( n = 5 \)) using clamp studies and OGTT (15). Correlations between clamp results and IS_{ogtt} were in the range of 0.6–0.8, depending on glucose tolerance and time in pregnancy, and correlations between these dynamic methods performed...
better than basal indices. We are not aware of any studies with validation of β-cell function indices during pregnancy.

Former investigations with indices of β-cell function and insulin sensitivity are mostly from mid-pregnancy and are not prospective. A cross-sectional study of pregnant Japanese women at three points in gestation demonstrated lower basal insulin sensitivity in GDM and overweight NGT women compared to normal weight NGT women (27). Only GDM subjects had significant declines in insulin sensitivity during gestation. HOMA-B levels in normal weight NGT subjects increased during pregnancy; however, in overweight and GDM subjects there was no increase in the last trimester. In our cohort, insulin sensitivity decreased during pregnancy in both NGT and GDM2. In GDM1, we only found a trend possibly due to the lifestyle intervention in this group.

As to our β-cell function data, AUCins/gluc increased in both normal and overweight NGT subjects, but in GDM subjects, only overweight gravida increased their β-cell function during gestation. These results, when compared to the Japanese results, may differ due to ethnicity, which is also relevant for the comparison of BMI groups. In subjects with GDM1, where a stronger β-cell defect is expected, the scarcity of cases in this cohort could influence our ability to detect changes in β-cell function indices.

Early onset of GDM (weeks 16–20) was found in 40% of subjects with GDM and normal BMI when tested with 75 g OGTT (4). In the early-onset group, significant reductions in β-cell function were found, as well as decreases in insulin sensitivity. Additionally, this group demonstrated a defect in compensation for IR, whereas β-cell function adjusted for insulin sensitivity in GDM subjects with a late onset did not differ from that in NGT subjects.

Pregnant Caucasian women who were referred for 100 g OGTT after positive glucose challenge tests (weeks 24–28) had impaired β-cell function relative to their degree of glucose intolerance based on the Stumvoll phase 1 and the ISogtt indices (19). We have calculated the same composite (with Stumvoll phase 1 index and ISogtt) from our dataset; results similar to the AUCh/s/gluc composite, for both the cohort and the subgroups, emerged. Correlation analysis of these two composites from our cohort resulted in $r=0.70–0.93$ (data not shown).

The verification of several β-cell function and insulin sensitivity indices as components of a hyperbolic relationship based on OGTT data, i.e. an oral DI, was recently done in NGT and GDM women post partum (17). In this cohort, only the combination of ISogtt and AUCh/s/gluc fit the strict hyperbolic criteria in both NGT and IGT subjects. We are not aware of similar data in pregnant women, but the above cohort encompasses a broad range of glycemias, i.e. women of childbearing age with NGT, IGT and diabetes, covering the range of glycemias found in our cohort.

The natural course of dysglycemia in our GDM1 subjects may have been obscured by mandatory lifestyle intervention after the diagnosis of GDM. The arrested deterioration in most indices from weeks 14–16 to 30–32 in this group, in comparison with GDM2 subjects, may reflect that lifestyle intervention after the diagnosis of GDM. The arrest deterioration in most indices from weeks 14–16 to 30–32 in this group, in comparison with GDM2 subjects, may reflect that lifestyle intervention was effective (28).

The HAPO study recently demonstrated that the risk of adverse pregnancy outcome increases continuously over the whole range of nondiabetic glucose levels (12). Our prospective data outline an impairment of β-cell function in the preclinical stages of GDM, which is masked by decreasing insulin sensitivity in pregnancy. In overweight women, this impairment is exacerbated, presumptively increasing the risk for adverse pregnancy outcomes and earlier transition to overt diabetes after pregnancy.

### Conclusion

We have found deterioration of β-cell function adjusted for insulin sensitivity in glucose-tolerant women and more so in glucose-intolerant women in the course of pregnancy. This failure to compensate decreases in insulin sensitivity was accentuated in both early and late pregnancies in overweight women.

### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
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**References**