Early Detection of Insulin Sensitivity and β-Cell Function with Simple Tests Indicates Future Derangements in Late Pregnancy


Department of Medical and Surgical Sciences (A.L., M.G.D., M.M., D.F.), University of Padova, 35100 Padova, Italy; Department of Gynecology, Perinatology and Human Reproduction (G.Mel., E.P., R.C.), and Laboratory (A.O., G.Mes.), Careggi Hospital; University of Florence, 4–50121 Florence, Italy; and Metabolic Modeling Unit, Institute of Biomedical Engineering (A.M., G.P.), and Aging Branch Institute of Neuroscience (C.M.), National Research Council, I-35127 Padova, Italy

Objective: Insulin sensitivity and secretion during early and late pregnancy were assessed in women with normal glucose tolerance and gestational diabetes mellitus (GDM).

Research Design and Methods: The oral glucose tolerance test (OGTT) was performed in 903 women at 16–20th gestational week, of whom 37 had GDM (GDM1 group), and 859 repeated the OGTT at wk 26–30. At the second test, 55 had GDM (GDM2 group); the others remained normotolerant (ND group). Insulin sensitivity from OGTT (as quantitative insulin sensitivity check index and OGTT insulin sensitivity) and β-cell function (as the ratio of the areas under the insulin and glucose concentration curves, adjusted for insulin sensitivity) were assessed in both tests.

Results: In early pregnancy the quantitative insulin sensitivity check index was not different in the three groups, whereas OGTT insulin sensitivity was lowest in GDM2, intermediate in GDM1, and highest in ND. In late pregnancy both indices were reduced in GDM compared with ND and lower than in early pregnancy. In early pregnancy GDM1, but not GDM2, had lower β-cell function than ND. During the late visit, GDM2 also showed impaired β-cell function compared with ND; furthermore, the adaptation to the increase to insulin resistance from early to late pregnancy was defective in GDM2.

Conclusions: In early pregnancy insulin sensitivity, as assessed from the OGTT but not from fasting measurements, is impaired in women who developed GDM. β-Cell function impairment is evident only when GDM is manifest and is characterized by inappropriate adaptation to the pregnancy induced increase in insulin resistance. (J Clin Endocrinol Metab 93: 876–880, 2008)

Normal pregnancy can be considered a state of insulin resistance because insulin sensitivity decreases in pregnancy, reaching its nadir in the third trimester and rapidly returning to prepregnancy levels after delivery (1–4). Although the specific mechanisms behind the progressive insulin resistance during pregnancy have not been completely clarified, an important contribution seems to come from the endocrine modifications characteristic of pregnancy, including the increase in estrogens, progesterone, human placental lactogen, cortisol, and TNF-α (5, 6). Both insulin resistance (7) and a defective insulin secretion and action (8, 9) also characterize gestational diabetes mellitus (GDM).

The euglycemic glucose clamp, the “gold standard” for assessing insulin sensitivity, has been used in pregnancy, but the test is complex, so most of the studies were performed on a small number of patients (1–3). Studies on insulin secretion, which usually simply evaluated the area under the concentration curve (1, 2), only rarely also using the glucose clamp (9), have involved a small number of patients, who were obese in most cases (1, 9). Evaluation of insulin sensitivity and secretion in large numbers

Abbreviations: AUC, Area under the curve; AUCglu, area under the glucose concentration curve; AUCins, area under the insulin concentration curve; AUCR, area under the curve ratio; FPG, fasting plasma glucose; FPI, fasting plasma insulin; GDM, gestational diabetes mellitus; ND, normotolerant; OGIS, oral glucose tolerance test insulin sensitivity; OGTT, oral glucose tolerance test; QUICKI, quantitative insulin sensitivity check index.
of subjects, or in particular conditions such as in pregnancy, can be done only with simple tests. The oral glucose tolerance test (OGTT) is definitely "easier" to perform than the glucose clamp, can be applied in large populations, and provides indices that have already been applied with success for studies in pregnancy, though in a limited number of subjects (e.g. 10, 11).

The aim of the present study was the evaluation of insulin sensitivity and secretion in a large population of pregnant women with normal glucose tolerance and GDM with a view of elucidating possible differences between early and late pregnancy in the overall metabolic state. In particular, we aimed to verify whether women who develop GDM in an early stage show the same defects in insulin secretion and sensitivity of those that develop GDM in a late phase. Knowing at the beginning of pregnancy the modifications of either insulin sensitivity or insulin secretion or both can help strategies that aim to normalize early the intrauterine metabolic milieu in a critical period for fetal metabolic imprinting (12). In addition, we also aimed to verify if surrogate indices of insulin sensitivity and secretion can be used to characterize metabolic conditions in the early and late pregnancy. For this purpose, both fasting and dynamic insulin sensitivity indices were derived from the OGTT, and β-cell function was assessed to evaluate the ability of the β-cells to compensate for changes of insulin sensitivity by modulating insulin secretion (13, 14).

### Subjects and Methods

#### Subjects and tests

From the population of pregnant women tested for GDM with a 75-g 2-h glucose load at the Perinatal Medicine Unit of the University of Florence between 1997 and 1999, subjects meeting the following inclusion criteria were invited to take part in this longitudinal study: Caucasian ethnicity, nulliparity, singleton pregnancy, absence of chronic hypertension, pregestational body mass index between 19 and 25 kg/m², and no specific conditions known to affect glucose metabolism. Informed consent was obtained from all subjects before they took part in the study that was conducted according to the Helsinki Declaration. GDM was diagnosed with an OGTT (75 g glucose), interpreted according to the Carpenter and Coustan criteria (15) and following the recommendations of the Fourth International Workshop Conference on Gestational Diabetes Mellitus (16). The 75-g glucose was assumed after a 10- to 12-h overnight fast; serum samples for glucose and insulin measurements were drawn at 0, 60, and 120 min.

A total of 903 women who underwent an OGTT between the 16th and 20th week of gestation (early pregnancy) was included in this study; 37 were diagnosed as affected by GDM, and 866 had a normal glucose tolerance. Of these normotolerant (ND) women, 859 repeated the OGTT between the 26th and 30th week (late pregnancy), and among them 55 were diagnosed as affected by GDM, whereas 804 remained ND. Therefore, we identified three separate groups: GDM at the early visit (GDM1); ND at the early visit and GDM at the late visit (GDM2); and ND at the early visit who remained so at the late visit (ND). Therefore, data of ND women at the early visit were retrospectively analyzed using the separate groups. Values of fasting plasma glucose (FPG) and insulin (FPI) in the three groups for the two visits are shown in Table 1. Maternal characteristics are shown in Table 2.

#### Assays calculations and statistics

Plasma glucose levels were measured with the glucose-oxidase method (17) and plasma insulin levels with a double antibody RIA method (18). The quantitative insulin sensitivity check index (QUICKI) was calculated as $1/\log(FPI) + \log(FPG))$, where FPG and FPI are the fasting glucose and insulin concentrations. The QUICKI generally provides a partial estimate of body insulin sensitivity because it mainly reflects changes in hepatic insulin sensitivity (20, 21). It is known that approximately 80% of insulin-dependent glucose disposal occurs in the periphery, in both normal and diabetic conditions (22), so the impaired insulin-mediated glucose disposal also in pregnancy should be reflected mainly by a greater impairment in peripheral than in hepatic insulin sensitivity (2). That is why we also assessed dynamic insulin sensitivity using the OGTT insulin sensitivity (OGIS) model (23), which measures glucose clearance during an oral challenge, as extensively validated vs. the glucose clamp in various pathophysiological conditions (20, 23, 24).

A recent study from DeFronzo and colleagues (20) in fact showed that OGIS correlates strongly with total glucose disposal, as assessed by the glucose clamp, whereas the homeostasis model assessment (from which the QUICKI directly derives) correlates with the basal hepatic insulin resistance. Thus, in our study we used the QUICKI as fasting insulin sensitivity index and OGIS as dynamic (postprandial) insulin sensitivity.

Insulin secretion was evaluated with the area under the insulin concentration curve (AUCins), whereas β-cell function (i.e. how β-cells respond to the glucose stimulus) was assessed as the ratio of AUCins to AUCglu, that is the area under the glucose concentration curve (i.e. $\text{AUCR} = \text{AUC}_{\text{ins}}/\text{AUC}_{\text{glu}}$). The β-cell function index AUCR is expected to be inversely related to insulin sensitivity (OGIS). Therefore, differences in β-cell function were assessed by testing if the curves relating AUCR to OGIS were the same in the compared groups. This test was performed by analysis of covariance, with AUCR as the independent variable and OGIS as the covariate. When the power to detect differences

### Table 1. FPG and FPI in GDM patients and ND pregnant women in early and late pregnancy

<table>
<thead>
<tr>
<th></th>
<th>Early</th>
<th>Late</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FPG (mg/dl)</td>
<td>FPI (µU/ml)</td>
</tr>
<tr>
<td>ND</td>
<td>74.4 ± 1.1</td>
<td>6.35 ± 6.68</td>
</tr>
<tr>
<td>GDM1</td>
<td>78.9 ± 8.6</td>
<td>6.65 ± 3.75</td>
</tr>
<tr>
<td>GDM2</td>
<td>77.3 ± 8.4</td>
<td>6.65 ± 3.75</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± sd. GDM1 patients are those found with GDM already at the early visit and not investigated afterwards. GDM2 patients are those found with GDM at the late visit.

- $a P = 0.00015$ (ND vs. GDM1).
- $b P = 0.00039$ (ND vs. GDM2).
- $c P < 0.0001$ (ND: early vs. late).
- $d P = 0.0043$ (ND vs. GDM2).
- $e P = 0.04$ (GDM2: early vs. late).
- $f P = 0.026$ (GDM2: early vs. late).
Results

In the early stage, fasting insulin sensitivity (QUICKI) was not different in the three groups (Table 3). At the late visit, both the ND and the GDM2 group exhibited a reduced QUICKI compared with the early visit but were only marginally (P = 0.05) different from each other. A different picture was observed when considering the dynamic OGIS. Already at the early visit, OGIS was lower in ND women who then developed GDM (GDM2) compared with those who remained ND and higher than in GDM1 (Table 3). At the late visit, OGIS in both groups was lower than at the early visit, and the difference between ND and GDM2 was highly significant (P < 0.0001). GDM1 exhibited higher insulin release compared with ND, but a lower β-cell function (ratio of insulin to glucose AUC). GDM2 had higher secretion as well but still had a β-cell function comparable to that of ND.

Concerning the analysis of the relationship between insulin secretion and insulin sensitivity, during the early visit, the inverse relationship between log(AUCR) and log(OGIS) was not different between the ND women and those who converted to GDM (Fig. 1; r = 0.51; P < 0.0001; for the group P = 0.0023). Therefore, in GDM1 but not in GDM2 the defect in compensation for insulin resistance was already detectable in the early stage of pregnancy. During the late visit, the inverse relationship between sensitivity and secretion was shifted downwards in GDM2 (r = 0.57; P < 0.0001; for the group P = 0.0003).

Finally, when the log-transformed ratio of β-cell function between the late and early visit was compared with the corresponding ratio of OGIS, an inverse relationship was observed between the two variables, but the relationship was flatter in the group of women who converted to GDM2 compared with ND (Fig. 2; r = 0.47; P = 0.0001 for the regression; P = 0.04 for the slope difference). This indicates that in the converters to GDM at the second visit the β-cell adaptation to the insulin sensitivity changes was impaired compared with women who remained ND.

Discussion

This longitudinal study in a large cohort of expectant mothers illustrates the differences in the behavior of metabolic parameters between normal and GDM women. Both groups were analyzed in the early and late stages of the pregnancy. Fasting insulin sensitivity, assessed using the QUICKI, decreased significantly in normal pregnant women with a normal body weight as the pregnancy progressed.

Table 2. Maternal characteristics (mean ± sd) of GDM and ND women

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ND (n = 811)</th>
<th>GDM (n = 92)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (yr)</td>
<td>31.4 ± 4.5</td>
<td>33.0 ± 4.0</td>
<td>0.11</td>
</tr>
<tr>
<td>Prepregnancy BMI (kg/m²)</td>
<td>22.5 ± 3.0</td>
<td>22.4 ± 3.5</td>
<td>0.87</td>
</tr>
<tr>
<td>Weight gain during pregnancy (kg)</td>
<td>13.4 ± 5.6</td>
<td>12.6 ± 6.4</td>
<td>0.39</td>
</tr>
</tbody>
</table>

BMI, Body mass index.

* Student’s t test and χ², two-way, as appropriate.

Table 3. Parameters related to insulin secretion and β-cell function (mean ± sd)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ND</th>
<th>GDM2</th>
<th>GDM1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early visit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUCins</td>
<td>4663 ± 2309</td>
<td>5460 ± 3149</td>
<td>6541 ± 2517</td>
</tr>
<tr>
<td>AUCins/AUCgluc</td>
<td>0.47 ± 0.22</td>
<td>0.44 ± 0.22</td>
<td>0.39 ± 0.16</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.40 ± 0.06</td>
<td>0.40 ± 0.06</td>
<td>0.38 ± 0.05</td>
</tr>
<tr>
<td>OGIS</td>
<td>468 ± 68</td>
<td>441 ± 60</td>
<td>406 ± 46</td>
</tr>
<tr>
<td>Late visit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUCins</td>
<td>6245 ± 3013</td>
<td>7619 ± 5027</td>
<td>8048 ± 2517</td>
</tr>
<tr>
<td>AUCins/AUCgluc</td>
<td>0.55 ± 0.27</td>
<td>0.47 ± 0.28</td>
<td>0.40 ± 0.16</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.39 ± 0.06</td>
<td>0.37 ± 0.04</td>
<td>0.38 ± 0.05</td>
</tr>
<tr>
<td>OGIS</td>
<td>432 ± 60</td>
<td>396 ± 57</td>
<td>412 ± 50</td>
</tr>
</tbody>
</table>

ND are normotolerant women at both visits (n = 804). GDM2 women were found with GDM at the late visit (n = 55). GDM1 women were found with GDM at the early visit (n = 37). AUC insulin: μU/ml h; AUCins/AUCgluc: 10² μU/ml/min/gluc; QUICKI: dimensionless; OGIS: ml/min/m². ND parameters of the late visit are different from the corresponding parameters of the early visit (P < 0.0001). For GDM2, AUC insulin of the late visit is higher (P < 0.00001) than at the early visit, whereas both the QUICKI (P < 0.001) and OGIS (P < 0.00001) are lower. GDM1 did not undergo the second visit.

Comparison with ND: *P < 0.02; **P < 0.0001; "<th> P < 0.03; "d P < 0.005.

Comparison of GDM1 vs. GDM2: *P < 0.005.

Comparison with ND: †P < 0.002; ‡P < 0.05.
This progressive deterioration in insulin sensitivity in normal pregnancy was demonstrated in a clamp study by Catalano et al. (1), who found a 56% reduction in insulin sensitivity in the third trimester of pregnancy in normal-weight pregnant women and a 47% reduction in obese women. Buchanan et al. (3) studied normal-weight and obese pregnant women with and without GDM, showing a 60–80% reduction in insulin sensitivity in the third trimester. This is considered a physiological mechanism to facilitate the supply of glucose to the fetus (25), and there is a parallel progressive increase in insulin secretion to maintain a normal glucose tolerance (4). Our investigation is the first to confirm this behavior of insulin sensitivity and secretion in quite a large number of normal pregnant women with normal body weight, and introduces the novelty of evaluating insulin sensitivity in women with and without GDM as their pregnancy progresses using simple, reproducible, and inexpensive tests. In late pregnancy, the QUICKI for the GDM women decreased much more than in women who remained ND, showing a faster deterioration in the liver’s sensitivity as they neared their term.

However, the results of our study show that basal indices in early pregnancy are unable to reveal the insulin sensitivity impairment in those GDM patients with a normal body weight; these indices instead can be used in late pregnancy, when the insulin sensitivity impairment peaks (4). To our knowledge, our present study is the first one that, by using oral glucose sensitivity indices, found differences between basal and dynamic insulin actions in pregnancy, suggesting a possible different glucose handling behavior between the liver and the periphery.

Later on, both the QUICKI and OGIS were reduced in GDM, showing an overall increase in insulin resistance at any level. Both indices provide precious information for characterizing insulin sensitivity in GDM women with a normal body weight at various stages of pregnancy. Our results on insulin sensitivity are consistent with those of Homko et al. (9), who used an index calculated during the last hour of the hyperglycemic clamp test and found a 27% lower β-cell function in seven GDM patients than in eight ND women. Similar results were obtained by modeling the iv glucose test in 10 normal-weight GDM women (10). Consequently, our results are not biased by the test used.

In addition to a marked insulin resistance (7, 26), GDM women are also characterized by a defective insulin secretion (8–10), but in early pregnancy we found no difference in fasting insulin in our groups; insulin AUC during the OGTT was even higher in GDM. In late pregnancy, basal insulin levels increased significantly in ND and GDM2 but remained similar in the two groups. Insulin AUCs were also higher in late pregnancy in both groups, but the increase in GDM2 led to significantly higher values than in ND. Despite the increased secretion, β-cell function did not change during the early phase, and it remained similar in the two groups in the late phase too. This is because the increased secretion coincided with a similar increase of plasma glucose. However, the 17% increase in β-cell function in ND later in pregnancy was significant, whereas the increase was less pronounced in GDM2 (13%) and failed to reach significance. From this point of view, it is worth analyzing the relationships between insulin secretion and sensitivity, which represent the β-cell’s ability to compensate for the increased insulin resistance by modulating insulin secretion. The important new finding in our population is that in the early phase, the β-cell compensation was still intact, even in those women who later developed GDM. Women who already had GDM at the first visit exhibited, on the contrary, a significant defect of this compensatory mechanism. During the late visit, this mechanism deteriorated in those women who were ND earlier developed GDM.

GDM is a complex pathophysiological condition, and among normal weight women with GDM, those who developed this condition earlier showed a more pronounced defect of insulin secretion with respect to those who had GDM in late pregnancy. Our findings allow a better understanding of the physiology of GDM, and can help with designing and implementing clinical strategies for pregnant and postpartum women. In fact, the presence of insulin resistance already in the early phase of pregnancy in those women who later developed GDM underlines the importance of interventions aimed to increase insulin sensitivity, e.g. excessive weight gain should be avoided to circumvent fur-
ther worsening of insulin resistance in late pregnancy that is related to the nutrients available for the fetus and, thus, to fetal growth (25). However, even in normal weight women with early onset of GDM a careful follow-up after pregnancy is recommended because the early defect of β-cell compensation likely increases their risk of type 2 diabetes. Therefore, monitoring insulin secretion and resistance would be essential to guide any therapeutic strategies to prevent fetal and maternal complications (27).

To this aim, the routine use of simple, reproducible, and inexpensive tests during pregnancy will definitely help. Although these simple methods have many advantages, we cannot deny possible limitations. Even if widely validated against the gold standards, the indices used in our study are nonetheless surrogate indices that provide an approximate, though reliable, figure of the physiological processes. Another aspect worth noting is that β-cell secretion has been evaluated from systemic insulin without C peptide. To the best of our knowledge, there is no study showing marked changes of hepatic insulin clearance in pregnancy: therefore, peripheral insulin should reflect β-cell release.

In conclusion, our study suggests that fasting measurements in pregnant women can provide a basis for a simple assessment of impaired insulin sensitivity, but dynamic indices (e.g. OGIS) are more sensitive to detect early and subtle differences in those who will develop GDM in the late stage of pregnancy. In addition, the dynamic indices allow the characterization of the compensatory mechanisms of β-cell function in response to changes in insulin resistance. Until now, there are no published recommendations for evaluation and management of pregnant women and their babies based on measurements of insulin sensitivity. Because the OGTT is used for diagnosis and clinical care, performing this test does not add further discomfort. Finally, the use of simple, reproducible, and inexpensive tests as routine tools for insulin sensitivity and secretion in pregnant women will help clinicians to better characterize maternal and fetal status, and guide any related clinical decisions.

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Address all correspondence and requests for reprints to: Annunziata Lapolla, M.D., Department of Medical and Surgical Sciences, Via Giustiniani n 2, 35100 Padova, Italy. E-mail: annunziata.lapolla@unipd.it.

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