Clinically Useful Estimates of Insulin Sensitivity During Pregnancy

Validation studies in women with normal glucose tolerance and gestational diabetes mellitus

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OBJECTIVE — We examined whether selected indexes of insulin sensitivity derived from an oral glucose tolerance test (ISOGTT) or fasting glucose/insulin levels (ISQUICKI and ISHOMA) can be used to predict insulin sensitivity in women before and during pregnancy.

RESEARCH DESIGN AND METHODS — A 2-h euglycemic-hyperinsulinemic clamp (5 mmol/l glucose, 40 mU·m−2·min−1 insulin) and a 120-min oral glucose tolerance test (75 g load pre gravid, 100 g pregnant) were repeated on 15 women (10 with normal glucose tolerance [NGT] and 5 with gestational diabetes mellitus [GDM]) pre gravid and during both early (12–14 weeks) and late (34–36 weeks) pregnancy. An index of insulin sensitivity derived from the clamp (ISCLAMP) was obtained from glucose infusion rates adjusted for change in fat-free mass and endogenous glucose production measured using [6,6-2H2]glucose.

RESULTS — Univariate analysis using combined groups and periods of pregnancy resulted in significant correlations between ISCLAMP and ISOGTT (r = 0.74, P < 0.0001), ISQUICKI (r = 0.64, P < 0.0001), and ISHOMA (r = 0.53, P < 0.0001). The ISOGTT provided a significantly better correlation (P < 0.0001) than either ISQUICKI or ISHOMA. Multivariate analysis showed a significant group effect (P < 0.0003) on the prediction model, and separate equations were developed for the NGT (r = 0.64, P < 0.0001) and GDM (r = 0.85, P < 0.0001) groups. When subdivided by period of pregnancy, the correlation between ISCLAMP and ISOGTT pre gravid was r = 0.63 (P = 0.0002), during early pregnancy was r = 0.80 (P < 0.0001), and during late pregnancy was r = 0.64 (P = 0.0002).

CONCLUSIONS — Estimates of insulin sensitivity from the ISOGTT during pregnancy were significantly better than from fasting glucose and insulin values. However, separate prediction equations are necessary for pregnant women with NGT and women with GDM.

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A number of standard clinical procedures are available for evaluating maternal insulin sensitivity during pregnancy, including the euglycemic-hyperinsulinemic clamp, the oral glucose tolerance test (OGTT), the intravenous glucose tolerance test, the Minimal Model, and various derivations of fasting glucose and insulin levels. The euglycemic-hyperinsulinemic clamp is considered by many the “gold standard” among these procedures (1). Although the clamp method can provide a precise measure of insulin sensitivity under physiological conditions, it is a relatively complicated and labor-intensive procedure and is not suitable for large-scale clinical or epidemiological studies. Therefore, a simple but valid estimate of insulin sensitivity is desirable to monitor and possibly reduce the potential adverse effects associated with hyperinsulinemia and/or hyperglycemia during pregnancy.

In a recent report, Matsuda and DeFronzo (2) validated an index of insulin sensitivity estimated from glucose and insulin levels during an OGTT (ISOGTT) against the clamp procedure. Katz et al. (3) have also validated an index of insulin sensitivity based on the mathematical relation between fasting insulin and glucose (ISQUICKI) against the clamp. The widely used homeostasis model of assessment (HOMA), originally proposed by Matthews et al. (4), is also based on a single glucose and insulin value (ISHOMA). Although these models are by no means a complete listing of possible alternative estimates of insulin sensitivity, they are representative of potentially important and relatively easily measured options that have been validated against the clamp procedure. The purpose of the present study was to determine whether the ISOGTT, the ISQUICKI, and the ISHOMA indexes could be used to accurately estimate insulin sensitivity during pregnancy among women with normal glucose tolerance (NGT) and those with gestational diabetes mellitus (GDM).

RESEARCH DESIGN AND METHODS — A total of 15 women volunteered to participate in the study. The protocol was approved by the Institutional Review Board for Human Subjects, and all volunteers gave informed consent in accordance with the MetroHealth Medical Center guidelines for the protection of human subjects. Euglycemic-hyperinsulinemic clamps, OGTTs, and body composition measurements...
were performed on three occasions on each subject: pregravid and during both early (12–14 weeks) and late (34–36 weeks) pregnancy. Clamp and body composition data on these subjects has been reported previously (5,6).

Body composition
Body density was determined by hydrostatic weighing after an overnight fast, according to the method described by Catalano et al. (7). Residual lung volume was determined during immersion by open-circuit nitrogen washout and fat-free mass (FFM) was estimated according to Keys and Brozek (8). The FFM data was used instead of body weight in the calculation of IS_{CLAMP} because skeletal muscle accounts for most glucose uptake during this procedure.

Euglycemic-hyperinsulinemic clamps
Single-stage euglycemic-hyperinsulinemic clamps were performed as described originally by DeFronzo et al. (1). After a 10- to 12-h overnight fast, the subjects voided morning urine and were weighed. A polyethylene catheter was inserted into an antecubital vein for infusion of insulin, glucose, and [6,6-2H₂]glucose (Cambridge Isotope Laboratories, Andover, MA). A second polyethylene catheter was inserted retrograde into a dorsal vein of the hand, and the hand was warmed in a heated box (65°C) for sampling of arterialized venous blood. Endogenous glucose output was measured using a primed constant infusion of [6,6-2H₂]glucose infused at a rate of 0.133 mEq/min and an enrichment intended to achieve ~1.0 mol percent excess for all subjects. Blood samples for endogenous glucose production were collected before starting the tracer infusion, at 10-min intervals during the last 30 min of the baseline period and the last 40 min of the clamp. After the baseline period, a primed continuous infusion (40 mU · m⁻² · min⁻¹) of human insulin (Humulin; Eli Lilly & Co., Indianapolis, IN) was initiated and maintained for a period of 2 h. Plasma glucose levels were clamped at 5.0 mmol/l during hyperinsulinemia by use of a variable glucose infusion (20% dextrose). Blood samples for plasma glucose and insulin determination were collected at 5- and 10-min intervals, respectively, during the clamp.

OGTs
Pregravid, a 75-g OGGT was performed on all subjects; during early and late pregnancy, the subjects were given a 100-g OGGT. The OGGT was performed after a 10- to 12-h overnight fast. Venous blood samples for glucose and insulin determination were drawn in the fasting state and at 30, 60, 90, and 120 min after ingestion of the glucose drink. Glucose tolerance in the nonpregnant state was classified according to the National Diabetes Data Group criteria (9). Glucose tolerance during pregnancy was defined according to the criteria of Carpenter and Coustan (10). Subjects were instructed to eat a diet consisting of at least 60% of energy as complex carbohydrate, 25% as fat, and 15% as protein for the week before the test.

Analytical procedures
Plasma glucose concentrations were measured by the glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Blood samples for insulin measurements were centrifuged at 4°C, and the plasma was stored at −70°C for subsequent analysis in duplicate by a double-antibody radioimmunoassay as previously described (6).

The [6,6-2H₂]glucose in the plasma samples was isolated by ion-exchange chromatography. A penta-acetate derivative of glucose was prepared according to Tseng and Kalhan (11). Plasma enrichment was determined using a gas chromatograph–mass spectrometer (Model 5985B; Hewlett-Packard, Palo Alto, CA).

Calculations and statistical analysis
The insulin concentration achieved during the clamp was defined as the mean of the values obtained during the last 40 min of the procedure. The amount of glucose infused was calculated for each 10-min interval and averaged for the last 40-min period. This value was used to estimate glucose disposal as glucose uptake in peripheral tissues under steady-state conditions. Endogenous glucose output during the clamp was estimated by the addition of a known amount of labeled glucose to the 20% glucose infusion. The addition of tracer glucose to the 20% glucose helps maintain steady-state enrichment and reduces the likelihood of calculating a negative endogenous glucose output (12). The glucose turnover was calculated as

\[ P = F \cdot (100/E) - 1, \text{ where } P \text{ is the turnover rate in mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}, E \text{ is the isotope enrichment, and } F \text{ is the infusion rate in mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}. \]

The insulin sensitivity index from the OGGT was calculated according to three different equations. The first is an equation derived by Matsuda and DeFronzo (2) in which insulin sensitivity is estimated by dividing a constant (10,000) by the square root of the product of fasting plasma glucose (FGP) times fasting plasma insulin (FPI) times the mean glucose (G) times mean insulin (I).

\[ IS_{OGGT} = \frac{10,000}{\sqrt{(FPG \cdot FPI)} \times (G \cdot I)} \]

The second equation is a more recently published equation by Katz et al. (3) named QUICKI (quantitative insulin sensitivity check index). QUICKI is the inverse log sum of fasting insulin (I₀) and fasting glucose (G₀).

\[ IS_{QUICKI} = \frac{1}{\log(I₀) + \log(G₀)} \]

For the third calculation, we used the HOMA equation developed by Matthews et al. (4). HOMA is derived from the product of the FPG and the FPI divided by a constant (22.5), assuming that normal young subjects have an insulin resistance of 1.

\[ IS_{HOMA} = \frac{(FPG \cdot FPI)}{22.5} \]

All values are presented as means ± SEM. Differences between dependent variables were examined with two-way analysis of variance. Specific mean differences were identified with a Scheffe’s post hoc test. The relation between insulin sensitivity measured during the clamp and estimated from the various equations was based on univariate and multivariate correlation analysis. Differences between r values were assessed using a percentile method bootstrap technique as previously described (3). The data were analyzed using the Statview II statistical package (Abacus Concepts, Berkeley, CA). The α-level for statistical significance was set at 0.05.
Indexes of insulin sensitivity during pregnancy

Table 1—Glucose and insulin metabolism measured using euglycemic-hyperinsulinemic clamps, OGTTs, and fasting glucose and insulin levels in women pregravid and during both early and late pregnancy

<table>
<thead>
<tr>
<th></th>
<th>Pregravid</th>
<th>Early pregnancy</th>
<th>Late pregnancy</th>
</tr>
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<tbody>
<tr>
<td>ISCLAMP (10^{-2} mg kg^{-1} • FFM min^{-1} • uU/ml)</td>
<td></td>
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<tr>
<td>NGT</td>
<td>14.2 ± 1.1*</td>
<td>16.4 ± 1.6††</td>
<td>9.7 ± 0.7*††</td>
</tr>
<tr>
<td>GDM</td>
<td>8.8 ± 2.5</td>
<td>9.8 ± 2.0</td>
<td>4.9 ± 0.8</td>
</tr>
<tr>
<td>Glucose area under the response curve (mg/dl • min)</td>
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<tr>
<td>NGT</td>
<td>4,022 ± 825*</td>
<td>5,659 ± 695*</td>
<td>9,022 ± 537††</td>
</tr>
<tr>
<td>GDM</td>
<td>7,371 ± 1,299</td>
<td>9,391 ± 988</td>
<td>11,826 ± 711†</td>
</tr>
<tr>
<td>Insulin area under the response curve (mU/ml • min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NGT</td>
<td>5,854 ± 832*</td>
<td>7,833 ± 1,560*</td>
<td>14,263 ± 1,714††</td>
</tr>
<tr>
<td>GDM</td>
<td>14,573 ± 4,605</td>
<td>18,834 ± 3,655</td>
<td>29,512 ± 8,684*</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
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</tr>
<tr>
<td>NGT</td>
<td>91.8 ± 1.3*</td>
<td>85.2 ± 1.2†</td>
<td>81.0 ± 1.0††</td>
</tr>
<tr>
<td>GDM</td>
<td>98.0 ± 1.8</td>
<td>87.4 ± 2.9††</td>
<td>88.6 ± 4.5††</td>
</tr>
<tr>
<td>Fasting insulin (uU/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NGT</td>
<td>8.6 ± 0.8*</td>
<td>7.6 ± 1.5</td>
<td>11.3 ± 1.3††</td>
</tr>
<tr>
<td>GDM</td>
<td>18.9 ± 4.1</td>
<td>16.8 ± 5.8</td>
<td>27.5 ± 5.6††</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 10 NGT, n = 5 GDM). Fasting glucose and insulin measurements were obtained before the OGTT. *Significantly different from the GDM group, (P < 0.05); †significantly different from the pregravid levels (P < 0.05).

RESULTS—Ten of the women had NGT, and five had GDM. The GDM group had a higher pregravid BMI (30.8 ± 2.8 kg/m², P < 0.04) than the NGT group (23.5 ± 1.8 kg/m²). There was no difference in age (31.4 ± 1.4 and 29.4 ± 1.8 years for NGT and GDM, respectively) or parity between the groups.

Insulin sensitivity measured during the clamp was higher pregravid and during pregnancy in the NGT group than in the GDM group (Table 1). Insulin sensitivity was reduced in both groups during late pregnancy compared with pregravid. Area under the glucose and insulin response curve was lower for the NGT group than for the GDM group. During pregravid, early pregnancy, and late pregnancy, fasting glucose and insulin were higher in the GDM subjects than in the NGT group.

Univariate analyses (Table 2) revealed that the strongest correlative measure was between ISCLAMP and ISOGTT (r² = 0.74, P < 0.0001), followed by ISQUICKI (r² = 0.64, P < 0.0001) and then ISHOMA (r² = 0.53, P < 0.0001). The ISQUICKI and ISHOMA were very closely correlated with each other (r² = 0.82, P < 0.0001). When the model analysis for ISCLAMP and ISOGTT was extended to include group as an independent predictor, the correlation was significantly increased (r² = 0.90, P < 0.0001). Consequently, separate regression lines are presented for the NGT and GDM groups (Fig. 1).

During the pregravid period, the overall correlation between the ISOGTT and the ISCLAMP was r² = 0.63 (P = 0.0002). When group was added as an independent variable, the correlation increased further to r² = 0.77 (P < 0.0001). The correlation between the ISCLAMP and the ISQUICKI was also significant, r² = 0.65 (P = 0.0001). The ISHOMA provided a correlation that was similar to both of the other indexes, r² = 0.66 (P < 0.0001). There was no difference in the predictive ability of all three indexes.

During early pregnancy, the overall correlation between the ISOGTT and the ISCLAMP improved to r² = 0.80 (P < 0.0001). The addition of group as an independent variable to the multivariate analysis yielded a correlation of r² = 0.87 (P < 0.0001). During early pregnancy, the correlation between the ISCLAMP and both the ISQUICKI (r² = 0.71, P < 0.0001) and the ISHOMA (r² = 0.52, P = 0.002) remained significant. The correlation for ISOGTT was significantly better (P < 0.0001) than either ISQUICKI or ISHOMA, and ISQUICKI provided a stronger correlation than ISHOMA (P < 0.0001).

In late pregnancy, there was a decrease in insulin sensitivity in both groups, and the correlations for this period were slightly lower than what was
observed for pregravid and early pregnancy. The correlation between $\text{ISOGTT}$ and $\text{ISCLAMP}$ was $r^2 = 0.63$ ($P = 0.0002$). Once again, multivariate analysis showed that group was an independent variable ($r^2 = 0.70$, $P < 0.0008$). The correlation between $\text{ISCLAMP}$ and both $\text{ISHOMA}$ ($r^2 = 0.61$, $P = 0.0003$) and $\text{ISQUICKI}$ ($r^2 = 0.64$, $P = 0.0001$) was also significant. At this time point, the correlation for $\text{ISOGTT}$ and $\text{ISHOMA}$ was significantly better ($P < 0.0001$) than for $\text{ISQUICKI}$.

**CONCLUSIONS** — It has been suggested that aberrant insulin sensitivity may be one of the primary mechanisms by which fetal metabolism is programmed to predispose individuals to obesity and type 2 diabetes in later life (13). An accurate and easy-to-measure index of insulin sensitivity could greatly contribute to the assessment of intrauterine growth and reduce the risk of these potentially negative programming outcomes. In the present study, we found that $\text{ISOGTT}$, $\text{ISQUICKI}$, and $\text{ISHOMA}$ were significantly correlated with a direct measurement of insulin sensitivity using the euglycemic-hyperinsulinemic clamp, before and during pregnancy. Although all three indirect methods were significantly correlated with insulin sensitivity from the clamp, the $\text{ISOGTT}$ provided the strongest index, followed by $\text{ISQUICKI}$ and then $\text{ISHOMA}$.

Direct measurement of insulin sensitivity using the euglycemic-hyperinsulinemic clamp is complex and requires multiple blood sampling; however, the indirect estimates used in this study are based on easy-to-measure OGTTs and/or fasting glucose and insulin levels (2–4). The $\text{ISOGTT}$ index is calculated from glucose and insulin measurements obtained during a standard 2-h OGTT. One of the main strengths of the equation is that it considers both hepatic and peripheral insulin sensitivity and attempts to account for pre- and postabsorptive states. Matsuda and DeFronzo (2) validated the index against the euglycemic-hyperinsulinemic clamp in a large sample of subjects spanning a wide range of age and obesity. The correlation was strongest among subjects with NGT ($r^2 = 0.53$) and weakest among those with type 2 diabetes ($r^2 = 0.30$). In the present study, when insulin sensitivity calculated from the $\text{ISOGTT}$ for all of the combined periods and subject groups was correlated with direct measurement from the clamp, the relation ($r^2 = 0.74$) was stronger than the original observations of Matsuda and DeFronzo (2). To check the clinical application of the $\text{ISOGTT}$, we ran a back extrapolation on several sets of data substituting the estimated $\text{ISOGTT}$ into the regression equation. None of the means were significantly different, e.g., all subgroups and times (11.6 ± 0.009 estimated, 11.6 ± 0.008 measured), all subjects, late pregnancy (8.1 ± 1.1 estimated, 8.1 ± 0.8 measured), NGT late (10.3 ± 1.1 estimated, 9.7 ± 0.7 measured), and GDM late (3.6 ± 0.3 estimated, 4.9 ± 0.8 measured). We ran an analysis of variance on the data estimated from the $\text{ISOGTT}$ and found the same differences between groups and over time as was found with the $\text{ISCLAMP}$ and reported in Table 1.

We also performed separate analysis by subgroup and found that both NGT and GDM groups provided correlations that were similar to the overall combined group. However, the correlation for the GDM group tended to be stronger than for women with NGT and was consistently higher at each period of pregnancy. This observation differs somewhat from that of Matsuda and DeFronzo (2), who found a stronger correlation among insulin-sensitive subjects compared with subjects with type 2 diabetes. One explanation for these differences may be found in the contrasting insulin secretory responses among women with GDM when compared with individuals with type 2 diabetes. It is known that with advancing gestation, the insulin secretory response to glucose increases. It is not clear whether women with GDM have a greater or lesser response than women with NGT. Data from our group show that obese women with GDM have a greater second-phase insulin secretory response than women with NGT (6). However, Buchanan et al. (14) have reported a reduced insulin response during late pregnancy among women with GDM compared with normal control subjects. In the present study, women with GDM had a higher insulin response to the OGTT than women in the NGT group. In contrast, impaired insulin secretion is a hallmark among patients with overt type 2 diabetes (15). Therefore, it seems that stronger correlations between $\text{ISCLAMP}$ and $\text{ISOGTT}$...
are obtained when the insulin response is robust. Overall, however, the index provided an excellent estimate of insulin sensitivity and may provide a useful tool for the assessment of changing insulin status during pregnancy.

The ISQUICKI, recently proposed by Katz et al. (3), is based on a logarithmic and reciprocal transformation of a single fasting glucose and insulin value. The model is very similar to HOMA and differs only in the treatment of the data. The ISQUICKI has been validated against the isoglycemic-hyperinsulinemic clamp and was found to have a good linear correlation ($r^2 = 0.61$). In the present study, insulin sensitivity assessed using the ISQUICKI also showed a strong linear correlation with direct assessment using the clamp. The strength of the relation was sustained when we examined the discrete time points before and during pregnancy. However, the ISQUICKI was slightly less robust in predicting insulin sensitivity than the ISOGTT. Overall, the primary difference between the ISQUICKI and the ISOGTT estimates is that the ISOGTT may provide more information on peripheral insulin sensitivity. This is partly because the ISOGTT accounts for insulin-mediated glucose uptake after ingestion of a glucose load. Nevertheless, ISQUICKI may provide an excellent alternative for assessing insulin sensitivity when glucose clamps and/or OGTTs are not practical. One of the most obvious advantages in using the ISQUICKI may be in clinical situations in which only a single blood sample is available or in large-scale clinical trials and epidemiological studies.

The HOMA model was the third index of insulin sensitivity used in the present study. The index is based on the premise that circulating glucose and insulin levels are determined by a feedback loop between the liver and pancreatic $\beta$-cells (16). Therefore, when glucose levels increase after a meal, a signal is sent to the pancreas and insulin is released from the $\beta$-cell. The model has been widely used for many years and has been shown to correlate well with insulin sensitivity measured using the insulin clamp procedure (17,18). A criticism of the model is its deviation from linearity with increasing insulin resistance; consequently, it is believed to be an inaccurate index for those with advanced type 2 diabetes (3). To understand why this may be the case, one must consider that severe insulin resistance, i.e., GDM and type 2 diabetes, is most often associated with the loss of both peripheral and hepatic insulin sensitivity. Because HOMA may be more reflective of changes in hepatic insulin sensitivity, it is not surprising that it generally provides a weaker estimate of total body insulin sensitivity. In the present study, the overall insulin sensitivity derived from HOMA correlated well with the direct assessment from the clamp. However, HOMA provided a weaker predictive index compared with ISOGTT and ISQUICKI. This may reflect the limitations of HOMA in detecting changes in peripheral insulin sensitivity with advancing gestation. A loss of peripheral insulin sensitivity has been noted in women with NGT and GDM during late pregnancy (6) and seems to be related to impaired insulin signaling in skeletal muscle (19). However, when we examined the data subdivided by period of gestation, ISQUICKI showed a strong correlation with insulin sensitivity measured directly with the clamp, even during late pregnancy, when insulin sensitivity was reduced. This may be related to the ability of ISQUICKI to detect the loss of hepatic insulin sensitivity, which also occurs with advancing gestation (6,20). Therefore, despite its possible limitations in assessing peripheral insulin sensitivity, ISQUICKI proved to be a good predictor of total insulin sensitivity throughout pregnancy and may be a useful tool for both clinicians and researchers who wish to assess maternal insulin status.

In conclusion, this study shows that of the indexes examined, the most accurate estimate of insulin sensitivity during pregnancy may be obtained from an OGTT. The ISQUICKI and ISHOMA indexes, which are based on a single blood sample, can also provide an easy but accurate measure of insulin sensitivity in pregnant women. Although these indexes are not intended to replace the clamp procedure in a research setting, in which precise quantitative changes in insulin sensitivity are important, they may provide clinicians and epidemiologists with a useful tool for assessing insulin sensitivity during pregnancy.

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References


