Estimates of Insulin Sensitivity Using Glucose and C-Peptide From the Hyperglycemia and Adverse Pregnancy Outcome Glucose Tolerance Test

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OBJECTIVE — To determine if glucose and C-peptide values obtained as part of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study could be used to estimate insulin sensitivity during late pregnancy.

RESEARCH DESIGN AND METHODS — A total of 78 women enrolled in the HAPO study were recruited for this ancillary study. Venous plasma samples were drawn after an 8- to 10-h fast (time 0) and at 30, 60, 90, and 120 min after a 75-g glucose challenge, which was performed at 24–32 weeks' gestation. Samples were analyzed for plasma glucose, insulin, and C-peptide. Insulin sensitivity was estimated using the established Matsuda and DeFronzo insulin sensitivity index for oral glucose tolerance tests (IS_{OGTT}). Insulin sensitivity was also calculated from two other commonly used indexes of insulin sensitivity (that for homeostasis model assessment [IS_{HOMA}] and that for quantitative insulin sensitivity check index [IS_{QUICKI}]). A new insulin sensitivity index was calculated using the glucose and C-peptide concentrations at 0 and 60 min to derive $IS_{HOMA\ C\text{-}pep}$, $IS_{QUICKI\ C\text{-}pep}$, and $IS_{OGTT\ C\text{-}pep}$. These indexes were then correlated with insulin sensitivity estimated from the IS_{OGTT}.

RESULTS — The strongest correlation with the IS_{OGTT} was obtained for $IS_{OGTT\ C\text{-}pep}$ ($r = 0.792$, $P < 0.001$). Further, the correlations of $IS_{HOMA\ C\text{-}pep}$ and $IS_{QUICKI\ C\text{-}pep}$ with IS_{OGTT} were also significant ($r = 0.676$, $P < 0.001$ and $r = 0.707$, $P < 0.001$, respectively).

CONCLUSIONS — These data suggest that calculated $IS_{OGTT\ C\text{-}pep}$ is an excellent predictor of insulin sensitivity in pregnancy and can be used to estimate insulin sensitivity in over 25,000 women participating in the HAPO study.

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Gestational diabetes mellitus (GDM) is a common metabolic disorder in developed countries occurring in 2–10% of pregnancies (1). GDM is associated with an increased risk of maternal and perinatal complications such as preeclampsia, macrosomia, shoulder dystocia, and neonatal hypoglycemia. Furthermore, the offspring of GDM pregnancies have an increased risk of obesity and type 2 diabetes in later life (2,3). In a recent study, Crowther et al. (4) reported that treatment of GDM in the form of dietary advice, blood glucose monitoring, and insulin therapy as required for glycemic control reduces the rate of serious perinatal complications. Although the risks associated with GDM are well recognized, there has been no general agreement about which glucose criteria should be used for diagnosis of GDM based primarily on maternal perinatal and neonatal outcomes.

The recently completed Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study was a prospective observational study during which >25,000 pregnant women in 10 different countries were recruited to determine the levels of glycemia, less severe than diabetes, associated with risks of large for gestational age, clinical neonatal hypoglycemia, cord-blood C-peptide, and primary cesarean delivery (5) as well as neonatal adiposity (6). The results confirm that there is a strong continuous association between maternal glucose concentrations below those currently used to diagnose GDM and adverse perinatal outcomes.

Normal human pregnancy is characterized by a significant decrease in insulin sensitivity (7). Furthermore, women developing GDM in addition to an inadequate insulin response have decreased insulin sensitivity compared with women with normal glucose tolerance (8). In many of these studies, insulin sensitivity has been estimated using a euglycemic-hyperinsulinemic clamp, which is considered the gold standard method. However, the clamp is a complicated, high-cost, and labor-intensive procedure and is not suitable for large studies.

Various investigators have validated indexes of insulin sensitivity derived from an oral glucose tolerance test (IS_{OGTT}) (9) or fasting glucose and insulin levels (that for homeostasis model assessment [IS_{HOMA}] and that for quantitative insulin sensitivity check index [IS_{QUICKI}]) (10,11): in all these studies, only nonpregnant adults were evaluated. However, we recently reported a study of normal glucose tolerant and GDM pregnant women, comparing different indexes of insulin sensitivity derived from OGTTs with hyperinsulinemic-euglycemic clamps throughout pregnancy (12). We concluded that during pregnancy, the IS_{OGTT} was the index that best correlated with insulin sensitivity when compared with the euglycemic-hyperinsulinemic clamp ($r = 0.86$, $P < 0.001$). Hence, the

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Table 1—*Demographics of study population*

Maternal	
Age (years)	27.6 ± 5.3
Height (cm)	163 ± 7
Pre-pregnancy weight (kg)	73.7 ± 20.3
Pre-pregnancy BMI (kg/m^2)	27.5 ± 7.0
Parity	
0	37
1	23
>1	18
Race/ethnicity	
Caucasian	58
African American	11
Hispanic	7
Asian	2

Data are means ± SD or *n*.

purpose of this study was to investigate if insulin sensitivity could be reasonably estimated using glucose and C-peptide measures obtained during a 75-g OGTT in HAPO study subjects.

RESEARCH DESIGN AND METHODS

— The protocol was approved by the MetroHealth's Institutional Review Board, and the Scientific Review Committee of the General Clinical Research Center (now the Clinical Research Unit of the Clinical and Translational Science Award) subjects were recruited from women who had already formally agreed to participate in the HAPO study. Each subject signed a written consent form describing the ancillary research protocol. None of the subjects or investigators was made aware of the results of the HAPO OGTT so as not to affect the outcome of the primary project, unless fasting or 2-h glucose values exceeded predefined thresholds. After completion of the HAPO study and publication of the primary results, a post hoc analysis was performed to assess the number of women in this ancillary study who would have been diagnosed as having GDM using criteria established by the Fourth International GDM Workshop criterion (13).

A total of 78 women enrolled in the HAPO study were evaluated as part of this ancillary study. None of the patients had medical or obstetrical problems in a previous or the index pregnancy. A 75-g HAPO OGTT was performed in all subjects as close as possible to the 28th week of gestation according to standardized procedures (14): mean ± SD gestational age at recruitment was 27.6 ± 1.2 weeks. The OGTT was performed after an 8- to 10-h overnight fast. The HAPO OGTT consisted of fasting and 30-, 60-, 90-, and 120-min glucose measures and a fasting and 60-min C-peptide determination. To calculate the IS_{OGTT}, plasma insulin was also obtained at fasting and at 30, 60, 90, and 120 min.

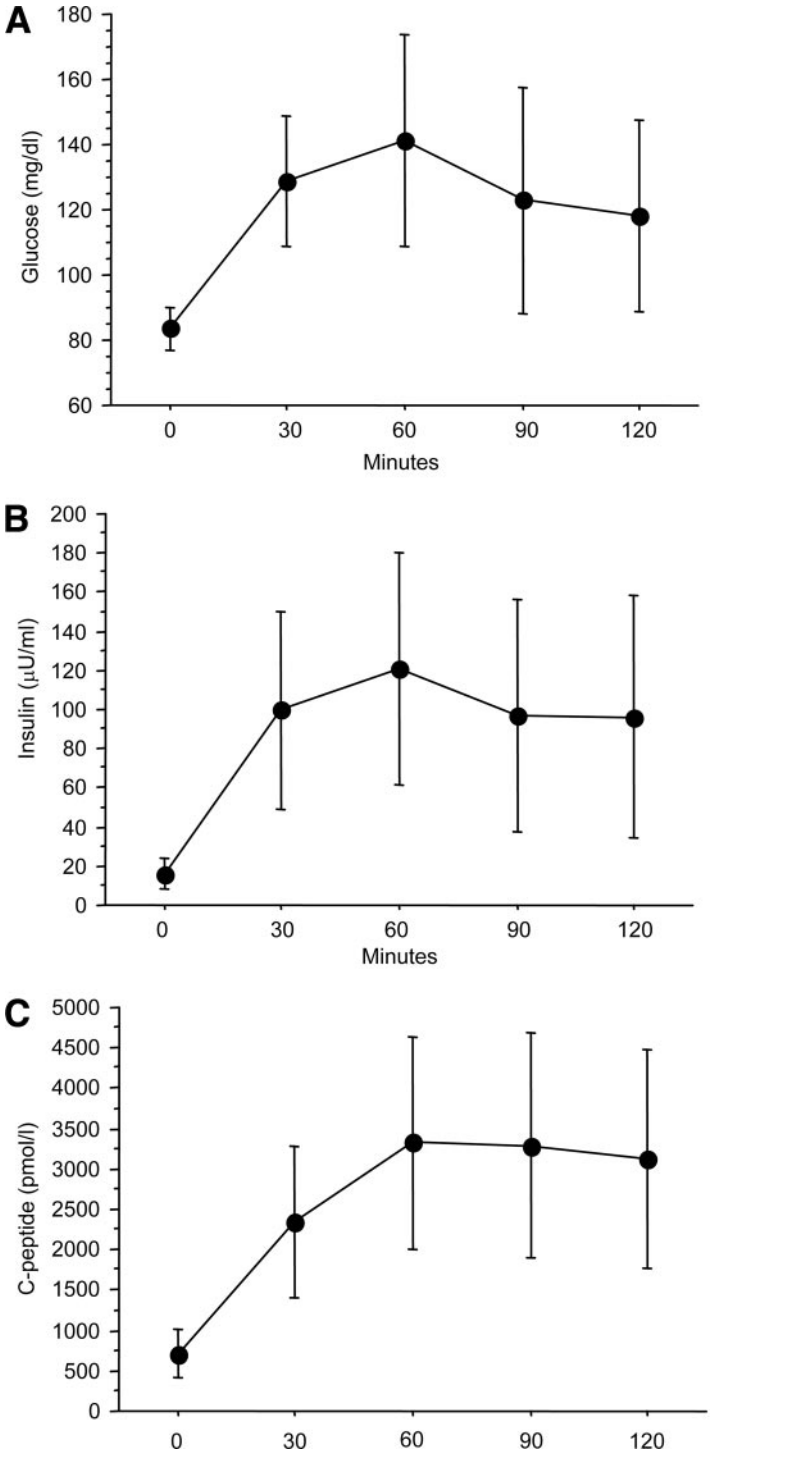

**Figure 1—*The results of the 75-g OGTT: A: Glucose.* B: *Insulin. C: C-peptide. Data are presented as means ± SD.* n = 78.

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 stored at −70°C. Insulin and C-peptide determinations were subsequently performed in duplicate on all samples by a double-antibody radioimmunoassay (Insulin: Linco, St. Louis, MO; C-peptide: Diagnostic Products, Los

Table 2—*Estimates of insulin sensitivity*

IS_{OGTT}	3.456 ± 1.678
$IS_{OGTT\ C\text{-}pep}$	5.016 ± 1.907
$IS_{HOMA\ C\text{-}pep}$	2,680 ± 1,293
$IS_{QUICKI\ C\text{-}pep}$	0.212 ± 0.009

Data are means ± SD.

Angeles, CA) as previously described (7). The glucose values are expressed in milligrams per deciliter, insulin as microunits per milliliter, and C-peptide as picomoles per liter.

The insulin sensitivity index was calculated from the OGTT according to three different equations. The first was the equation described by Matsuda and DeFronzo (IS_{OGTT}) (9): insulin sensitivity was calculated as follows:

$$IS_{OGTT} = 10{,}000/\sqrt{(FPG) \times (FPI)} \times (G \times I)$$

FPG and FPI are fasting plasma glucose and fasting plasma insulin, respectively, whereas *G* and *I* are mean glucose and mean insulin from 0 to 120 min.

The second equation was described by Matthews et al. (10) (IS_{HOMA}), and insulin sensitivity was calculated as follows:

$$IS_{HOMA} = 22.5/(FPG \times FPI)$$

The last considered equation was proposed by Katz et al. (11) and is called IS_{QUICKI} (Quantitative Insulin Sensitivity Check Index):

$$IS_{QUICKI} = 1/[\log(FPI) + \log(FPG)]$$

Insulin sensitivity indexes were then calculated using glucose and C-peptide concentrations available as part of the standard HAPO OGTT, i.e., C-peptide instead of insulin, at 0 and 60 min ($IS_{OGTT\ C\text{-}pep}$, $IS_{HOMA\ C\text{-}pep}$, and $IS_{QUICKI\ C\text{-}pep}$). To have the units from the estimates of insulin sensitivity from the $IS_{OGTT\ C\text{-}pep}$ resemble those of the IS_{OGTT}, 500,000 was used as the numerator rather than 10,000.

All data are presented as means ± SDs. Because IS_{OGTT} is the index that best correlated with insulin sensitivity calculated from the euglycemic-hyperinsulinemic clamp (12), we evaluated the correlation between $IS_{OGTT\ C\text{-}pep}$, $IS_{HOMA\ C\text{-}pep}$, and $IS_{QUICKI\ C\text{-}pep}$ with IS_{OGTT} using Pearson correlations and linear regression analyses. Comparisons between the normal glucose tolerant and GDM groups were made using Mann-Whitney *U* tests because of non-normal distribution of the data. All analyses were performed with Statistix, version 8.0 (Abacus Concepts, Berkeley, CA). A *P* value ≤0.05 was considered significant.

RESULTS — Maternal demographic characteristics of the study population are presented in Table 1. Mean maternal pregravid BMI was >26 kg/m^2, reflecting a tendency toward our study subjects being overweight and obese. The results of the

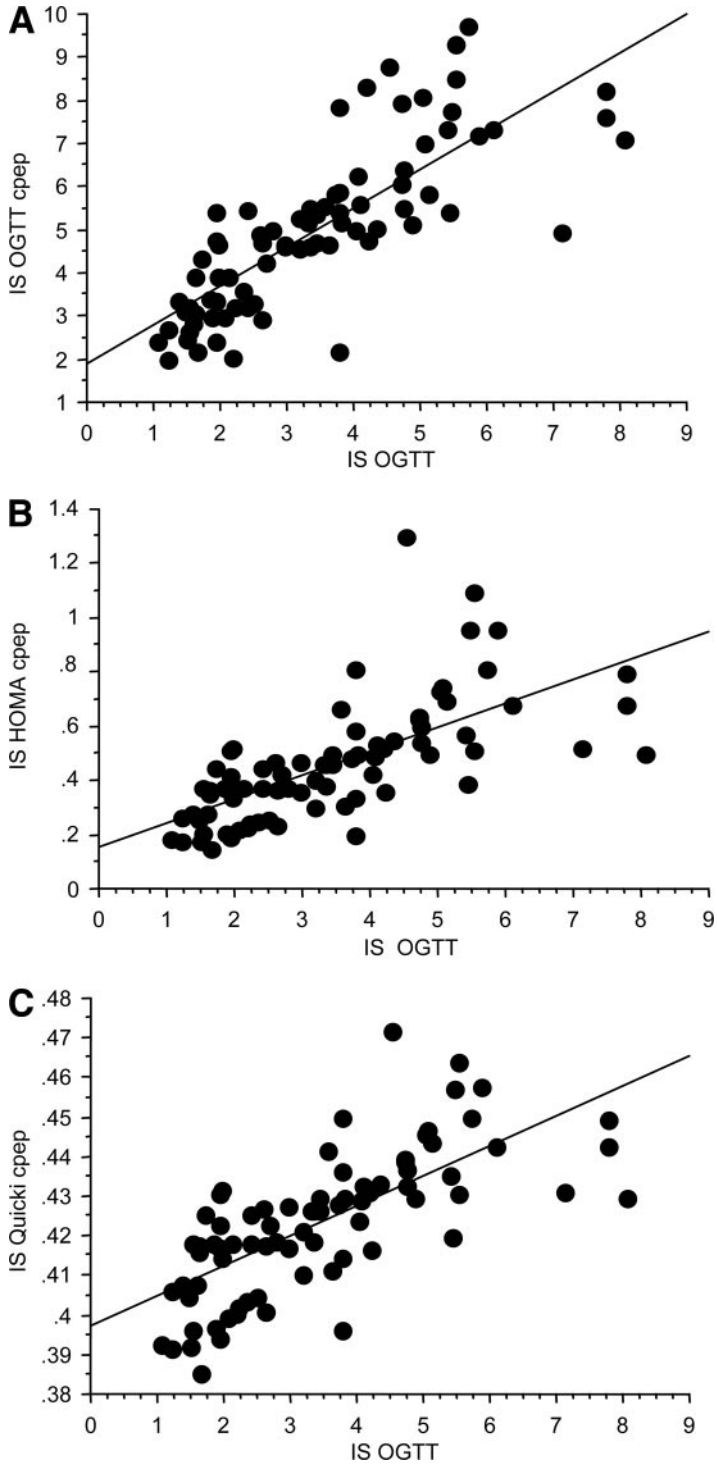

Figure 2—*The regression models for IS_{OGTT} and $IS_{OGTT\ C\text{-}pep}$ (A): y = 1.906 + 0.9*x, r = *0.792*, P < *0.001*; $IS_{HOMA\ C\text{-}pep}$ (B): y = *0.156 + 0.088*x, r = *0.676*, P < *0.001*; and $IS_{QUICKI\ C\text{-}pep}$ (C): y = *0.397 + 0.008*x, r = *0.707*, P < *0.001*, n = *78*.*

75-g OGTT are shown in Fig. 1: fasting glucose, post-OGTT glucose, insulin, and C-peptide levels are shown in Fig. 1*A*, *B*, and *C*, respectively. Mean fasting glucose was 83.5 ± 6.5 mg/dl and mean fasting insulin and C-peptide concentrations were 16.0 ± 8.1 μU/ml and 710 ± 304 pmol/l, respectively. Six of the 78 (7.7%) subjects had GDM as defined by the Fourth International Workshop Conference on GDM criteria (13). The mean ± SD estimates of insulin sensitivity for the IS_{OGTT}, $IS_{OGTT\ C\text{-}pep}$, $IS_{HOMA\ C\text{-}pep}$, and $IS_{QUICKI\ C\text{-}pep}$ are shown in Table 2.

The correlations between insulin sensitivity indexes ($IS_{OGTT\ C\text{-}pep}$, $IS_{HOMA\ C\text{-}pep}$, and $IS_{QUICKI\ C\text{-}pep}$) and IS_{OGTT} are shown in Fig. 2. $IS_{OGTT\ C\text{-}pep}$ ($r = 0.792$, $P < 0.001$) had the strongest correlation with the standard IS_{OGTT}. In contrast, using the fasting glucose and C-peptide data, the correlations of $IS_{HOMA\ C\text{-}pep}$ and $IS_{QUICKI\ C\text{-}pep}$ with IS_{OGTT} were $r = 0.676$ ($P < 0.001$) and $r = 0.707$ ($P < 0.001$), being weaker compared with $IS_{OGTT\ C\text{-}pep}$ (Fig. 2). There was a stronger association (multiple *r*) when a squared term was added to the regression model; $IS_{OGTT\ C\text{-}pep}$ ($r = 0.821$, $P < 0.0001$), $IS_{HOMA\ C\text{-}pep}$ (multiple $r = 0.705$, $P < 0.001$), and $IS_{QUICKI\ C\text{-}pep}$ ($r = 0.748$, $P < 0.001$), but the relative strength of the relationships to IS_{OGTT} was similar to the correlations from the simple linear models (Fig. 3).

Last, there was a significant inverse correlation between maternal prepregnancy BMI and $IS_{OGTT\ C\text{-}pep}$ ($r = -0.385$, $P < 0.001$), and the six women with GDM had decreased insulin sensitivity compared with women with normal glucose tolerance ($IS_{OGTT\ C\text{-}pep}$ 2.86 ± 1.16 vs. 5.20 ± 1.85, $P = 0.0025$).

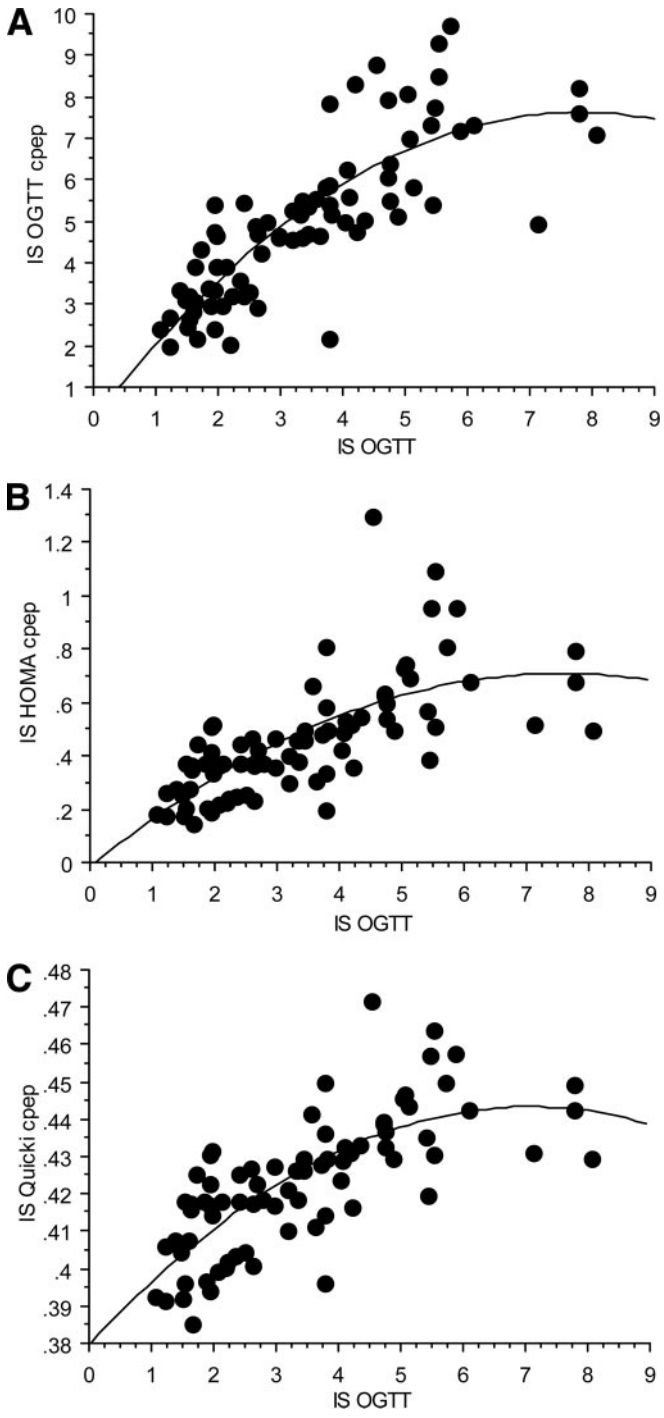

Figure 3—*The regression models for IS_{OGTT} and $IS_{OGTT\ C\text{-}pep}$ (A):* $y = 0.261 + 1.894x - 0.122x^2$, *multiple* $r = 0.821$; *$IS_{HOMA\ C\text{-}pep}$* (B): $y = -0.016 + 0.192x - 0.013x^2$, *multiple* $r = 0.705$; *and $IS_{QUICKI\ C\text{-}pep}$* (C): $y = 0.38 + 0.018x - 0.001x^2$, *multiple* $r = 0.748$, $n = 78$.

CONCLUSIONS— Measurement of insulin sensitivity in >25,000 HAPO subjects using either the euglycemic-hyperinsulinemic clamp or the intravenous minimal model technique was not possible. We therefore elected to use the IS_{OGTT} as our reference standard, based on previous work by Matsuda and DeFronzo showing that the IS_{OGTT} correlated well with the euglycemic clamp in a large number of study subjects having a wide range of age and body size (9). Furthermore, we have previously shown (12) that during pregnancy, the IS_{OGTT} had the strongest correlation with insulin sensitivity as measured by the euglycemic clamp, adjusted for residual hepatic glucose production. In this earlier study, the correlation of the IS_{OGTT} with insulin sensitivity in pregnancy measured by clamp was $r = 0.86$ ($P < 0.001$). The correlation during early pregnancy (12–14 weeks) was $r = 0.89$ ($P < 0.001$) and during late pregnancy (34–36 weeks) was $r = 0.80$ ($P < 0.001$). The correlation of the IS_{OGTT} with the clamp was also highly significant for both women with normal glucose tolerance and women with GDM. One of the strengths of the IS_{OGTT} as a measure of insulin sensitivity is that it incorporates both basal and hepatic measures of insulin sensitivity as well as post-absorptive or peripheral insulin sensitivity.

In the present study, the $IS_{OGTT\ C\text{-}pep}$ proved to be the best predictor of insulin

sensitivity when we used the standard IS_{OGTT} as the reference measure of insulin sensitivity. These data give us the confidence that the $IS_{OGTT\ C\text{-}pep}$ can provide important data regarding insulin sensitivity in the greater HAPO population and opens the possibility of developing large-scale metabolic studies from the data generated in this cohort. The $IS_{OGTT\ C\text{-}pep}$ may also be a useful tool because it includes the C-peptide response to glucose and hence provides an important measure of insulin secretion. As noted by Matsuda and DeFronzo (9), the IS_{OGTT} provides a more robust measure of insulin sensitivity in normoglycemic individuals compared with individuals with type 2 diabetes. This may be related to the greater insulin secretory capacity in normal glucose tolerant individuals. Because normal pregnancy is associated with an increase in insulin response (7), the inclusion of a measure of insulin secretion such as in the $IS_{OGTT\ C\text{-}pep}$ may be advantageous in assessing metabolic function in the HAPO cohort.

$IS_{HOMA\ C\text{-}pep}$ and $IS_{QUICKI\ C\text{-}pep}$ were also used to estimate insulin sensitivity in this study. IS_{HOMA} is frequently used to estimate insulin sensitivity in nonpregnant individuals. The index is based on the use of the fasting glucose and insulin. The index assumes that the circulating glucose and insulin are determined by a feedback loop between the liver and pancreatic β-cells. IS_{QUICKI} is based on a logarithmic and reciprocal transformation of a single fasting glucose and insulin value. The model is similar to a HOMA model and differs only in the treatment of the data. In contrast to IS_{OGTT}, IS_{HOMA} and IS_{QUICKI} incorporate only basal or fasting glucose and insulin measures and may be more reflective of only hepatic insulin sensitivity. Therefore, it is not surprising that $IS_{HOMA\ C\text{-}pep}$ and $IS_{QUICKI\ C\text{-}pep}$ provided a less robust estimate of insulin sensitivity compared with $IS_{OGTT\ C\text{-}pep}$. Supplementary Table 1, showing the correlations between $IS_{HOMA\ C\text{-}pep}$ and $IS_{QUICKI\ C\text{-}pep}$ in comparison with IS_{HOMA}, IS_{QUICKI}, and IS_{OGTT}, is provided in an online appendix, available at http://care.diabetesjournals.org/cgi/content/full/dc09-1463/DC1. We observed similar results when IS_{HOMA} and IS_{QUICKI} were compared with the clamp measures during pregnancy in our previous study.

A limitation of our protocol is that we estimated HAPO pancreatic β-cell function using C-peptide at time 0 and 60 rather than insulin. The reason for using C-peptide rather than insulin was that with over 25,000 maternal samples to be transported across continents, there was a risk that some samples might be hemolyzed. Hemolysis is known to increase insulin degradation but not to affect C-peptide (15). Therefore, because insulin and C-peptide are secreted in equimolar levels, we elected to use C-peptide rather than insulin to estimate insulin sensitivity.

In conclusion, this study confirms that among the three proposed indexes, the best estimate of insulin sensitivity during pregnancy in the HAPO study subjects can be obtained from the $IS_{OGTT\ C\text{-}pep}$. The index provides a reproducible and accurate measure of maternal metabolism during pregnancy. The ability to assess insulin sensitivity in a large and diverse subject population evaluated in the HAPO study may aid in the assessment of the short- and long-term affect of maternal metabolism on the offspring and the potential of fetal programming outcomes. Although these indexes cannot replace more direct measures of insulin sensitivity such as the euglycemic clamp, $IS_{OGTT\ C\text{-}pep}$ can be considered a reasonable low-cost clinical parameter for assessing insulin sensitivity during pregnancy in the 25,000 subjects who participated in the HAPO study.

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No potential conflicts of interest relevant to this article were reported.

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