Hormonal and Metabolic Factors Associated With Variations in Insulin Sensitivity in Human Pregnancy

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OBJECTIVE — The objective of this study was to determine maternal hormonal and metabolic factors associated with insulin sensitivity in human pregnancy.

RESEARCH DESIGN AND METHODS — This was a prospective observational cross-sectional study of 180 normal pregnant women, using samples collected at the time of a blinded oral glucose tolerance test (OGTT) between 24 and 32 weeks' gestation as an ancillary to the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study. The study was conducted at two public university teaching hospitals, Cleveland, Ohio, and Brisbane, Australia. Fasting maternal serum cholesterol, triglycerides, free fatty acids, insulin, leptin, tumor necrosis factor-α, placental growth hormone (PGH), insulin-like growth factors (IGFs) 1 and 2, and insulin-like growth factor binding proteins (IGFBPs) 1 and 3 were assayed. Correlation and multiple regression analyses were used to determine factors associated with maternal insulin sensitivity (IS) estimated using both OGTT-derived (IS_{OGTT}) and fasting (using the homeostasis model assessment [HOMA]; IS_{HOMA}) insulin and glucose concentrations.

RESULTS — Insulin sensitivity correlated (r = x and y for IS_{OGTT} and $IS_{HOMA,}$ respectively) with fasting maternal serum leptin (-0.44 and -0.52), IGFBP1 (0.42 and 0.39), and triglycerides (-0.31 and -0.27). These factors were significantly associated with insulin sensitivity in multiple regression analyses (adjusted R^2 0.44 for IS_{OGTT} and IS_{HOMA}). These variables explained more than 40% of the variance in estimates of insulin sensitivity.

CONCLUSIONS — Maternal hormonal and metabolic factors related to the placenta, adipose tissue, and the growth hormone axis are associated with the variation in insulin sensitivity seen during normal human pregnancy.

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The development of insulin resistance in pregnancy has been recognized for many years, but the causal mechanisms remain unclear. Ryan et al. (1) first demonstrated a 40% decrease in insulin sensitivity in women with gestational diabetes as compared with a control group at term. Later, Catalano at al. (2) confirmed these results describing longitudinal changes in insulin sensitivity and insulin response in women with normal glucose tolerance and gestational diabetes before and during pregnancy. Despite a general tendency to attribute whole-body insulin resistance in pregnancy to placental hormones (3), the precise contribution of various hormonal factors remains poorly defined. Human placental lactogen was an early candidate, although findings have been variable (4). Kirwan et al. (5) have suggested an important role for tumor necrosis factor (TNF)-α, whereas placental growth hormone (PGH) has been shown to induce insulin resistance in a mouse model (6) and to correlate with maternal glycemia in patients with diabetes (7). Our study was designed to further explore the maternal metabolic and hormonal correlates of insulin resistance in a healthy pregnant population. We hypothesized that factors in addition to placental hormones were associated with insulin resistance during normal pregnancy.

RESEARCH DESIGN AND METHODS
— The protocol was approved by the Hospital Institutional Review Board and the Scientific Review Committee of the General Clinical Research Center (GCRC) at Metro Health, Cleveland, Ohio, and by the Human Research Ethics Committee of Mater Health Services, South Brisbane, Australia. Both of these centers participated in the international multicenter Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study (8), and subjects consented in writing both to the main HAPO study and to this ancillary study. Subjects and investigators were blinded to the results of the oral glucose tolerance test (OGTT), so as not to affect the outcome of the primary HAPO project.

For this ancillary study 180 women enrolled in HAPO, including 80 from Cleveland, Ohio, were recruited. Their characteristics are shown in Table 1. A 75-g OGTT was performed after 8–10 h overnight fasting in all subjects between 24 and 28 weeks (as close as possible to the 28th week) of gestation according to standardized procedures. The OGTT consisted of 0- (fasting), 60-, and 120-

Table 1—*Maternal characteristics and biochemical variables*

Age at delivery (years)	29.1 ± 5.5
Prepregnancy weight (kg)	71.1 ± 18.6
Prepregnancy BMI (kg/m²)	26.2 ± 6.4
Gestation at OGTT (weeks)	27.9 ± 1.6
Weight at OGTT (kg)	81.3 ± 18.4
BMI at OGTT (kg/m²)	30.0 ± 5.9
IGFBP1 (nmol/l)	6.18 (3.30–5.69)
IGFBP3 (nmol/l)	141.50 (95.55–184.49)
PGH (ng/ml)	10.11 (5.94–12.48)
Cholesterol (mmol/l)	6.18 (5.52–6.82)
Triglycerides (mmol/l)	2.02 (1.56–2.28)
Leptin (ng/ml)	35.92 (21.01–45.43)
Free fatty acids (mmol/l)	0.59 (0.47–0.71)
TNF-α (pg/ml)	2.50 (0.87–2.83)
IGF1 (ng/ml)	164.32 (25.14–276.60)
IGF2 (ng/ml)	893.34 (770.69–1,050.41)

Data are means ± SD or median (interquartile range). Clinical characteristics of women who participated in the study (presented as means ± SD) were normally distributed. Biochemical and hormonal variables measured in the study (presented as median [interquartile range]) were nonnormally distributed and were transformed as natural logarithms for further analyses.

min glucose measures as well as fasting and 60-min C-peptide determinations. Ancillary study patients had estimations of serum insulin at 0, 60, and 120 min. As part of the HAPO protocol, subjects were unblinded if fasting plasma glucose level was >105 mg/dl (5.8 mmol/l), 2-h OGTT plasma glucose >200 mg/dl (11.1 mmol/l), or any recorded value <45 mg/dl (2.5 mmol/l). This study includes only women whose OGTT results were within HAPO limits. Three women would have been classified as having gestational diabetes by the National Diabetes Data Group criteria and eight by the Carpenter Coustan criteria (9). However, because all glucose results were blinded, we have not excluded these women from this report. Other hormonal and metabolic factors were measured in the fasting state.

Glucose assays in HAPO used the glucose oxidase method and were carefully standardized across all HAPO centers under the supervision of the central laboratory in Belfast, U.K. The other biochemical and hormonal assays for this ancillary study were performed at either the GCRC Cleveland (insulin, leptin, free fatty acids, TNF-α, and insulin-like growth factors [IGFs] 1 and 2) or Mater Health Services Brisbane labs (PGH, IGF binding proteins [IGFBPs] 1 and 3, cholesterol, and triglycerides) in one or two batches, with one shipment of samples in each direction. Samples with hemolysis were excluded prior to testing. All assays were performed in duplicate. Assay coefficients of variation (CVs) are shown in supplementary Table 1A in the online appendix, available at http://care.diabetesjournals.org/cgi/content/full/dc09-1196/DC1.

Insulin samples were centrifuged at 4°C and stored at −70°C. Insulin was assayed using a double-antibody radioimmunoassay (Linco, St. Charles, MO) as previously described (2). Leptin, free fatty acids, TNF-α, IGF1, IGF2, PGH, IGFBP1, and IGFBP3 were assayed using previously described methods (5,7,10).

Based on previous work by the Cleveland group (11) the insulin sensitivity (IS) index calculated from the OGTT (IS_{OGTT}) according to the equation first described by Matsuda and DeFronzo formed our primary measure of insulin sensitivity. Specifically, insulin sensitivity was calculated as follows: $IS_{OGTT} = 10,000/\sqrt{(FPG) \times (FPI) \times (G \times I)}$; where FPG and FPI are fasting plasma glucose (mg/dl) and insulin (μU/ml), respectively, and G and I are mean glucose and mean insulin, respectively, of all samples from 0–120 min. We also calculated the simpler homeostasis model assessment (HOMA) measure based on fasting samples only (IS_{HOMA}) (11). In this case, insulin sensitivity is calculated as $IS_{HOMA} = 405 / (FPG \times FPI)$.

Statistical methods
The distributions of all variables were tested using analysis of skewness and kurtosis. Maternal characteristics (supplementary Table 1A) and the dependent variables IS_{OGTT} and IS_{HOMA} were normally distributed, but all other biochemical variables required log transformation to approximate a normal distribution.

Natural logarithms have been used in further analyses.

We used linear product moment (Pearson) correlations followed by multiple linear regression analysis to explore the relationships between variables. Dependent variables were IS_{OGTT} and IS_{HOMA}. Independent variables included all measured maternal biochemical parameters, maternal prepregnancy BMI, BMI at the OGTT, age, and center (Cleveland vs. Brisbane). Results reported include standardized regression coefficients (β) with 95% CIs and partial correlation coefficients. STATA (StataCorp, College Station, TX) and Statistica (StatSoft, Tulsa, OK) were used for statistical analyses. Significance was accepted at the 5% level on two-tailed testing.

RESULTS — The characteristics (means ± SD) of the 180 women who participated in this study are outlined in Table 1. The median (interquartile range) for the biochemical and hormonal variables are also shown in Table 1. Only age at delivery differed between the Cleveland and Brisbane participants. Non-Hispanic whites were the predominant ethnic group (80%), with Hispanics 3%, Asians 9%, and other ethnicities 8%. The subjects' mean prepregnancy BMI was in the overweight range. Mean gestation at the time of OGTT was very close to the HAPO goal of 28 weeks. The Pearson correlation coefficients between maternal biochemical variables, estimates of insulin resistance, and maternal BMI (prepregnancy and at the OGTT visit) are shown in Table 2. BMI, IGFBP1, triglycerides, and leptin correlated significantly with the estimates of maternal insulin sensitivity.

Subsequently, multiple regression analyses were performed. Results are reported for IS_{OGTT} in Table 3. Maternal BMI calculated at the OGTT visit, although significant in simple correlations (Pearson $r = -0.47$, −0.48 for BMI vs. IS_{OGTT} and IS_{HOMA}, respectively), became not statistically significant after adjusting for the other variables in the model. Models incorporating prepregnancy BMI rather than BMI at the OGTT showed essentially the same findings. As can be seen from Table 3, the model incorporating all biochemical variables accounted for 44% of the observed variance in IS_{OGTT}. Multiple regression findings for IS_{HOMA} were virtually identical (multiple R^2 0.48, adjusted R^2 0.44; $P < 0.0001$) and are not shown separately. Leptin, IGFBP1, and triglycerides were

Table 2—Pearson correlation coefficients among insulin sensitivity estimates, BMI, and biochemical variables

	IS_{OGTT}	IS_{HOMA}	Prepregnancy BMI	BMI at OGTT visit
Prepregnancy BMI	−0.415*	−0.410*	1.000	0.940*
BMI at OGTT visit	−0.470*	−0.484*	0.940*	1.000
IGFBP1	0.421*	0.386*	−0.316*	−0.360*
IGFBP3	0.002	0.043	−0.0390	−0.039
PGH	−0.041	−0.005	−0.198†	−0.223*
Cholesterol	−0.047	−0.076	−0.123	−0.096
Triglycerides	−0.311*	−0.269*	0.159†	0.106
Leptin	−0.437*	−0.519*	0.448*	0.550*
Free fatty acids	−0.051	0.006	0.0462	0.052
TNF-α	−0.023	0.0390	−0.0128	−0.039
IGF1	−0.055	0.027	0.0434	0.053
IGF2	−0.104	−0.033	0.0550	0.036

Pearson correlation coefficients between calculated maternal BMI (prepregnancy and at the OGTT visit), biochemical, and hormonal parameters measured in the study (transformed to natural logarithms) and estimates of insulin sensitivity (IS_{OGTT} and IS_{HOMA}). *$P < 0.01$; †$P < 0.05$.

significantly related to both insulin sensitivity estimates. These findings were not altered by exclusion of those participants who would have been classified as suffering from gestational diabetes by the National Diabetes Data Group or Carpenter Coustan criteria.

To determine whether maternal overweight/obesity influenced the factors associated with insulin sensitivity, we repeated the regression analyses with participants characterized by prepregnancy BMI less than or more than 25 kg/m². Because the relationship between BMI and fat mass may vary across ethnic groups, we also repeated all analyses using only those participants from the dominant ethnic group (non-Hispanic whites). Both the stratified and non-Hispanic white only BMI analyses gave very similar results to those presented for the whole cohort, and the data are not presented separately. The other ethnic subgroups were considered too small for separate analysis. Differences in the relationship between BMI, adiposity, and leptin concentrations between ethnic groups may explain in part why leptin and not BMI has a stronger correlation with estimates of insulin resistance.

CONCLUSIONS — The current study demonstrates that a substantial proportion of the variance in maternal insulin sensitivity in pregnancy is associated with variations in maternal biochemical variables related to the placenta (leptin) adipose tissue (leptin and triglycerides) as well as the growth hormone axis (IGFBP1). The placenta is a major source of leptin in pregnancy and also the source of high concentration of PGH, which upregulates the growth hormone/IGF axis during pregnancy (7,12). Although leptin is produced both in placenta and adipose tissue, several lines of evidence suggest that the major changes in leptin during pregnancy relate to placental leptin production (13). First, maternal leptin decreases abruptly after delivery of the placenta. Second, there is no correlation between change in maternal BMI and leptin. Third, the pregnancy-related increase in maternal leptin predates increased fat mass in pregnancy (13). In a longitudinal study the Cleveland group (14) has also demonstrated a close correlation between serum leptin and fat oxidation during early and late pregnancy but not in the nonpregnant state. This provides a further mechanism by which leptin may influence maternal insulin sensitivity. Recent evidence demonstrates that maternal obesity also influences both placental and circulating monocyte/macrophage populations and inflammatory markers (15), suggesting that adipose and placental tissue contributions to the overall maternal metabolic and inflammatory milieu are interlinked.

Interestingly, the inverse relationship of maternal BMI to insulin sensitivity, well recognized in many studies, was no longer statistically significant in our model when the panel of 10 biochemical and hormonal variables were included. Although these parameters are, in themselves, significantly correlated with maternal BMI (Table 2), they appeared more strongly related to insulin sensitivity in the multiple regression analyses.

An etiologic role has been proposed for reduction in IGFBP1 as a link between maternal obesity and increased birth weight through increased bioactive IGF1 in maternal serum (16,17). Confirming these previous findings, our study demonstrated a negative correlation between maternal BMI and IGFBP1. Reduced IGFBP1 in women with higher BMI would be predicted to increase free maternal IGF1 and promote nutrient transfer to the fetus and fetal growth. Indeed, IGFBP1 has been reported to be negatively correlated with fetal lean body mass, though not with fat mass (18), suggesting a specific effect on fetal body composition.

PGH showed a weak negative corre-

Table 3—Regression model: dependant variable IS_{OGTT}

	β	95% CI (β)	Partial correlation	P
Leptin	−0.365	−0.535 to −0.195	−0.330	<0.001
IGFBP1	0.319	0.180 to 0.458	0.349	<0.001
Triglycerides	−0.293	−0.432 to −0.155	−0.327	<0.001
PGH	−0.136	−0.286 to 0.015	−0.146	0.076
BMI at OGTT visit	−0.142	−0.311 to 0.028	−0.135	0.100
Cholesterol	0.096	−0.045 to 0.238	0.110	0.181
Center	−0.160	−0.457 to 0.137	−0.088	0.288
Maternal age	0.063	−0.061 to 0.188	0.082	0.317
IGF1	−0.078	−0.359 to 0.203	−0.045	0.584
IGFBP3	−0.016	−0.206 to 0.174	−0.014	0.870
Free fatty acids	−0.006	−0.141 to 0.130	−0.007	0.935
TNF-α	0.003	−0.143 to 0.149	0.003	0.967
IGF2	−0.001	−0.179 to 0.176	−0.001	0.989

Summary of multivariable regression of biochemical and other parameters associated with estimates of insulin sensitivity (IS_{OGTT} and IS_{HOMA}). Standardized correlation coefficients (β) and their 95% CIs as well as partial correlations are shown for each variable. Overall multiple R^2 0.49; adjusted R^2 0.44; $P < 0.0001$.

lation with insulin sensitivity in the multiple regression analysis, but this failed to reach statistical significance ($P = 0.076$). A negative relationship of PGH with insulin sensitivity would be predicted from known growth hormone actions in the nonpregnant state and with findings of decreased insulin sensitivity related to elevated PGH in a transgenic mouse model (6). One previous study by Fuglsang et al. (19) also found no correlation between PGH and fasting insulin sensitivity estimated just prior to delivery. The effects of PGH thus appear (at best) modest in normal human pregnancy. Other factors are clearly of greater importance.

Previous findings regarding the relationship of maternal hormones and adipokines to insulin sensitivity have been variable. Using the frequently sampled intravenous glucose tolerance test (IVGTT) in a small group of patients ($n = 38$), McLachlan et al. (12) reported that leptin correlated negatively with insulin sensitivity, but adiponectin, TNF-α, and C-reactive protein proved unrelated to insulin sensitivity. In contrast, one previous report of 15 pregnant women using the insulin clamp (5) from our group noted TNF-α as a significant factor. However, subjects in that study included obese women with gestational diabetes who also had significantly elevated plasma TNF-α during pregnancy. Partitioning of TNF-α may also be of importance in this regard. The recent study from Challier et al. (15) demonstrated increased TNF-α in peripheral blood and placental mononuclear cells, associated with insulin resistance, in obese pregnant women, but no changes were noted in maternal plasma TNF-α. In the current study, we did not find any association of TNF-α with insulin sensitivity. A further recent study from Mastorakos et al. (20) confirmed a relationship between leptin and insulin resistance, reported no relationship of insulin resistance with adiponectin, and noted an association of insulin sensitivity with yet another adipocytokine, visfatin. The often divergent findings about relationships among adipocytokines, BMI, and insulin sensitivity are summarized in a recent review by Briana et al. (21).

Do the correlations described in our study represent underlying causes of variations in maternal insulin sensitivity in pregnancy or merely the consequences of such variations? A causal role seems possible for IGFBP1 as described above. Leptin has been noted to directly modulate insulin sensitivity in vitro (22) and has been described as a predictor of gestational diabetes independent of maternal BMI (23). Pregnancy is a physiological leptin-resistant state because increased maternal energy intake and positive energy balance develop in late pregnancy despite increased leptin levels, which would be predicted to reduce appetite and energy intake in a fully leptin-sensitive state (13). Leptin has also been reported to reduce insulin secretion in both rodent and human islets in vitro (24). However, the uniform hyperinsulinemia of normal pregnancy despite high leptin concentrations again suggests leptin resistance at the level of the β-cells. Partitioning effects may also be important for leptin because it has been noted that placental leptin mRNA and protein content is three- to fivefold higher in type 1 diabetic pregnancies than in those of control subjects, despite comparable maternal serum leptin concentrations (13).

In summary, our data demonstrate that variations in maternal insulin sensitivity in normal pregnancy relate in part to the maternal adipocytokine and growth hormone/IGF axes. Our findings are novel in that they extend the range of potential factors examined simultaneously in relation to maternal insulin sensitivity and include a much larger number of subjects than in most previous reports. We acknowledge that estimation of insulin sensitivity using the IS$_{OGTT}$ and IS$_{HOMA}$ is less precise than gold-standard measurement with an insulin clamp, but we would consider that clamp studies are not feasible in a cohort of this size. Further, we have established strong correlations with the clamp method in previous studies.

It is plausible, though not yet proven, that these systems serve to regulate whole-body insulin sensitivity in individual pregnant women. An improved understanding of these factors may potentially open new avenues of treatment in gestational diabetes and other conditions associated with insulin resistance in pregnancy, such as obesity and preeclampsia.

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