

TNF- α Is a Predictor of Insulin Resistance in Human Pregnancy

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Historically, insulin resistance during pregnancy has been ascribed to increased production of placental hormones and cortisol. The purpose of this study was to test this hypothesis by correlating the longitudinal changes in insulin sensitivity during pregnancy with changes in placental hormones, cortisol, leptin, and tumor necrosis factor (TNF)- α . Insulin resistance was assessed in 15 women (5 with gestational diabetes mellitus [GDM] and 10 with normal glucose tolerance) using the euglycemic-hyperinsulinemic clamp procedure, before pregnancy (pregravid) and during early (12–14 weeks) and late (34–36 weeks) gestation. Body composition, plasma TNF- α , leptin, cortisol, and reproductive hormones (human chorionic gonadotropin, estradiol, progesterone, human placental lactogen, and prolactin) were measured in conjunction with the clamps. Placental TNF- α was measured *in vitro* using dually perfused human placental cotyledon from five additional subjects. Compared with pregravid, insulin resistance was evident during late pregnancy in all women (12.4 ± 1.2 vs. 8.1 ± 0.8 10^{-2} $\text{mg} \cdot \text{kg}^{-1}$ $\text{fat-free mass} \cdot \text{min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}^{-1}$). TNF- α , leptin, cortisol, all reproductive hormones, and fat mass were increased in late pregnancy ($P < 0.001$). *In vitro*, most of the placental TNF- α (94%) was released into the maternal circulation; 6% was released to the fetal side. During late pregnancy, TNF- α was inversely correlated with insulin sensitivity ($r = -0.69$, $P < 0.006$). Furthermore, among all of the hormonal changes measured in this study, the change in TNF- α from pregravid to late pregnancy was the only significant predictor of the change in insulin sensitivity ($r = -0.60$, $P < 0.02$). The placental reproductive hormones and cortisol did not correlate with insulin sensitivity in late pregnancy. Multivariate stepwise regression analysis revealed that TNF- α was the

most significant independent predictor of insulin sensitivity ($r = -0.67$, $P < 0.0001$), even after adjustment for fat mass by covariance ($r = 0.46$, $P < 0.01$). These observations challenge the view that the classical reproductive hormones are the primary mediators of change in insulin sensitivity during gestation and provide the basis for including TNF- α in a new paradigm to explain insulin resistance in pregnancy. *Diabetes* 51:2207–2213, 2002

Pregnancy is a period marked by profound changes in a woman's hormonal status and metabolism. The ability to regulate nutrient balance during this period is critical to the health of the mother and the growing fetus. Insulin is one of the key regulators of metabolism, and significant changes in insulin sensitivity and its ability to control glucose, fat, and protein during pregnancy have been well documented (1–4). Previous reports have also shown that maternal insulin resistance plays an important role in the regulation of maternal energy metabolism, fat accretion, and fetal growth (5,6). In gestational diabetes mellitus (GDM), greater insulin resistance may lead to abnormal blood glucose and fetal macrosomia, may increase the likelihood of obstetric complications, and in some cases, may increase the risk of stillbirth. The cellular mediators of insulin resistance in late pregnancy have long been ascribed to alterations in cortisol and placental-derived hormones including human placental lactogen (HPL), progesterone, and estrogen (7–9). To our knowledge, however, the changes in insulin sensitivity during gestation have not yet been correlated with hormonal changes using a prospective longitudinal design.

Recently, investigators have focused on several new potential mediators of insulin resistance including the cytokine tumor necrosis factor (TNF)- α , the anti-obesity hormone leptin, and adipose-derived resistin, adiponectin, and free fatty acids (10,11). Among these candidates, TNF- α and leptin are known to be produced in the placenta and therefore could play a central role in insulin resistance during pregnancy (12–14). Although increased circulating TNF- α levels have been associated with insulin resistance in obesity, aging, sepsis, muscle damage, and preeclamptic pregnancy, reports of change in levels during normal pregnancy and GDM are equivocal (15–22). Furthermore, because TNF- α is synthesized and secreted from the placenta and adipose tissue, the origin of circulating levels during pregnancy remains largely unknown. Leptin was first identified as a product of the *ob* gene in

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FFM, fat-free mass; GDM, gestational diabetes mellitus; hCG, human chorionic gonadotropin; HPL, human placental lactogen; IRS, insulin receptor substrate; NGT, normal glucose tolerance; RIA, radioimmunoassay; TNF, tumor necrosis factor.

TABLE 1
Subject characteristics and glucose tolerance pregravid and during late pregnancy

	Lean NGT	Obese NGT	GDM
Age (years)	33 \pm 2	30 \pm 1	29 \pm 2
Parity	1.2 \pm 0.4	0.8 \pm 0.2	1.0 \pm 0.3
Weight (kg)	53.5 \pm 1.4	76.8 \pm 7.4*	83.3 \pm 9.4*
BMI (kg/m ²)	19.8 \pm 1.0	27.3 \pm 2.4*	30.8 \pm 2.8*
Body fat (%)	25.0 \pm 1.6	36.6 \pm 3.2*	40.1 \pm 3.2*
2-h Glucose, pregravid	102.6 \pm 17.8	106.6 \pm 2.8	141.0 \pm 6.5
2-h Glucose, during late pregnancy	125.0 \pm 8.3	145.6 \pm 8.1†	173.4 \pm 4.9*†

Data are means \pm SE; $n = 5$ per group. *Significantly different from lean NGT group, $P < 0.02$. †Significantly different from pregravid, $P < 0.03$.

adipose tissue and is thought to regulate energy balance through central hypothalamic pathways, but it may also play a role in insulin resistance by promoting lipid oxidation and inhibiting lipid synthesis (23–25). There are several reports of increased circulating leptin during pregnancy, due in part to placental secretion (13,14,26).

The primary purpose of this prospective study was to relate the longitudinal changes in concentrations of reproductive hormones (HPL, progesterone, human chorionic gonadotropin [hCG], prolactin, and estradiol), TNF- α , leptin, and cortisol with the corresponding changes in maternal insulin sensitivity as measured by the euglycemic-hyperinsulinemic clamp. To further elucidate the role of TNF- α as a mediator of maternal insulin resistance, we examined the placenta as a potential contributing source of maternal circulating TNF- α using the dual perfusion model of human placental cotyledon.

RESEARCH DESIGN AND METHODS

Subjects. Fifteen women (10 with normal glucose tolerance [NGT] and 5 who developed GDM) volunteered to participate in the longitudinal evaluation of insulin sensitivity during pregnancy (Table 1). These subjects are a subset of a larger study examining insulin sensitivity (5,27). Euglycemic-hyperinsulinemic clamps, fasting blood samples, and body composition measurements were performed on each subject, on three occasions: pregravid and during both early (12–14 weeks) and late (34–36 weeks) pregnancy. The women with GDM had abnormal glucose tolerance during the third trimester according to the criteria of Carpenter and Coustan (28). The protocol was approved by the Institutional Review Board for Human Subjects, and all volunteers signed an informed consent form in accordance with the MetroHealth Medical Center guidelines for the protection of human subjects.

Body composition. Body composition was determined by hydrostatic weighing and total body water using a two-compartment model according to the method described by Catalano et al. (29). Height was measured without shoes to the nearest 1.0 cm, and body weight was measured to the nearest 0.1 kg.

Euglycemic-hyperinsulinemic clamps. Single-stage euglycemic-hyperinsulinemic clamps were performed as described previously (30,31). Endogenous glucose output was measured using a primed constant infusion of [6,6-²H₂]glucose. Hyperinsulinemia was achieved using a primed-continuous infusion (40 mU \cdot m⁻² \cdot min⁻¹) of human insulin (Humulin; Eli Lilly, Indianapolis, IN) for a period of 2 h. Plasma glucose levels were clamped at 5.0 mmol/l using a variable glucose infusion (20% dextrose). Blood samples for plasma glucose and insulin determination were drawn at 5- and 10-min intervals, respectively, during the clamp.

Placental perfusions. Five human placentas from uncomplicated pregnancies were collected immediately after cesarean sections. Perfusion of a suitable placental lobule was carried out as described previously (32). The placenta was washed before perfusion, and the medium was recirculated in the fetal and maternal circulation for 120 min. The media on both circuits were continuously gassed with 95% O₂, 5% CO₂. The volumes of fetal (Vf) and maternal (Vm) perfusion media collected at the end of the washing and

recirculation period were recorded, and the media were stored at -20°C for later analysis. To estimate TNF- α release into the fetal ($Q_f = C_f \times V_f$) and maternal ($Q_m = C_m \times V_m$) circulation, TNF- α concentrations were measured in fetal (C_f) and maternal (C_m) media. These values were divided by the duration of the perfusion ($t = 120$ min) to give the rates of TNF- α release toward the fetal and maternal side of the placental circulation. TNF- α was also assessed on triplicate tissue samples in the placenta, before (Q_{ti}), and at the end of (Q_{te}) the perfusion to evaluate changes in placental TNF- α ($Q = Q_{te} - Q_{ti}$). The rate of placental TNF- α synthesis was estimated as $PLS = Q_f + Q_m + Q$ divided by the duration of the perfusion for one cotyledon, and as $TPS = PLS \times P/l$, where P/l is the placenta/lobule weight ratio, for the whole placenta.

Analytical procedures. All samples for TNF- α , leptin, cortisol, and the reproductive hormones were run in duplicate in a single assay. Plasma TNF- α concentrations were measured by enzyme-linked immunosorbent assay (Quantikine HS; R&D Systems, Minneapolis, MN). The intra-assay coefficient of variation was 14%, the minimum detectable limit of the assay was 0.18 pg/ml, and the lowest standard was 0.5 pg/ml. 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 by a double-antibody radioimmunoassay (RIA) as previously described (27). Plasma cortisol, estradiol, HPL, and progesterone were determined by RIA (Diagnostic Products, Los Angeles, CA). Plasma leptin samples were also measured by RIA (Linco Research, St. Charles, MO). Plasma hCG was determined by immunoradiometric assay (Diagnostic Products). Plasma prolactin was measured by RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA).

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 Tserng and Kalhan (33). Plasma enrichment was determined using a gas chromatograph mass spectrometer (model 5985B; Hewlett-Packard, Palo Alto, CA).

Calculations and statistical analysis. The insulin sensitivity index from the clamp procedure was estimated as the glucose infusion rate plus endogenous glucose output divided by the mean insulin concentration during the clamp and is expressed as 10^{-2} milligrams per kilogram fat-free mass (FFM) per minute per microunit per milliliter (10^{-2} mg \cdot kg⁻¹ FFM \cdot min⁻¹ \cdot μU^{-1} \cdot ml⁻¹).

All values are presented as means \pm SE. Differences between dependent variables were examined with two-way ANOVA. Specific mean differences were identified with a Scheffe post-hoc test. The relationship between insulin sensitivity measured during the clamp and estimated from the various equations was based on univariate and multivariate correlation analysis. 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.

RESULTS

Body weight and body composition responses. Mean age at entry into the study was 31 ± 1 years, and parity was 0.93 ± 0.15 . Body weight pregravid was 71.2 ± 5.0 kg and did not change markedly during early pregnancy (72.7 ± 5.0 kg) but, as expected, increased during late pregnancy (83.0 ± 5.1 kg). Fat mass was similar pregravid (25.7 ± 3.7 kg) and during early pregnancy (25.4 ± 3.3 kg) and increased during late pregnancy (28.3 ± 3.4 kg; $P < 0.0003$).

Insulin action. Pregravid, there was no difference in insulin sensitivity between the lean and obese women with NGT, but women who developed GDM were more insulin resistant than those with NGT (Table 2). During early pregnancy, insulin sensitivity increased slightly in all subgroups of women, and when the data were combined, there was a $\sim 14\%$ increase in sensitivity (Fig. 1). However, by late pregnancy, insulin sensitivity was reduced $\sim 65\%$ versus pregravid. All subgroups of women had similar relative changes in sensitivity regardless of whether they had NGT or GDM. Fasting glucose measurements declined during early and late pregnancy ($P < 0.05$) versus pregravid. In contrast, fasting insulin was significantly in-

TABLE 2

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

	Pregravid	Early pregnancy	Late pregnancy
IS _{CLAMP} (10 ⁻² mg · kg ⁻¹ · FFM · min ⁻¹ /μU · ml ⁻¹)			
Lean NGT	14.3 ± 1.3	17.4 ± 1.2	9.9 ± 0.5*
Obese NGT	14.2 ± 1.9	15.4 ± 3.1	9.5 ± 1.5*
Obese GDM	8.8 ± 2.5†	9.8 ± 2.0†	4.9 ± 0.8†*
Fasting glucose (mg/dl)			
Lean NGT	90.4 ± 2.0	83.6 ± 1.2*	81.0 ± 0.9*
Obese NGT	93.2 ± 1.7	86.8 ± 1.8	81.0 ± 1.9*
Obese GDM	98.0 ± 1.8	87.4 ± 2.9	88.6 ± 4.5*
Fasting insulin (μU/ml)			
Lean NGT	8.2 ± 0.9	6.1 ± 0.6	10.7 ± 0.9*
Obese NGT	9.0 ± 1.6	9.2 ± 3.0	11.9 ± 2.7*
Obese GDM	18.9 ± 4.1†	16.8 ± 5.8†	27.5 ± 5.6†*

Data are means ± SE; *n* = 5 per group. IS, insulin sensitivity derived from euglycemic-hyperinsulinemic clamp studies. *Significantly different from pregravid, *P* < 0.001. †Significantly different from lean NGT group, *P* < 0.05.

creased (*P* < 0.05) in late pregnancy compared with either pregravid or early pregnancy.

TNF-α and hormonal responses. The women showed a downward trend (13%) in TNF-α from pregravid (1.79 ± 0.27 pg/ml) to early pregnancy (1.56 ± 0.22 pg/ml) and a 45% increase in late pregnancy (2.59 ± 0.24 pg/ml; *P* < 0.004) (Fig. 2). These changes were consistent regardless of NGT or GDM status (Table 3). During late pregnancy, TNF-α levels were higher (*P* < 0.01) for women with GDM than for lean women with NGT.

Data obtained from the in vitro experiments showed that the rate of placental TNF-α production was 123.1 ±

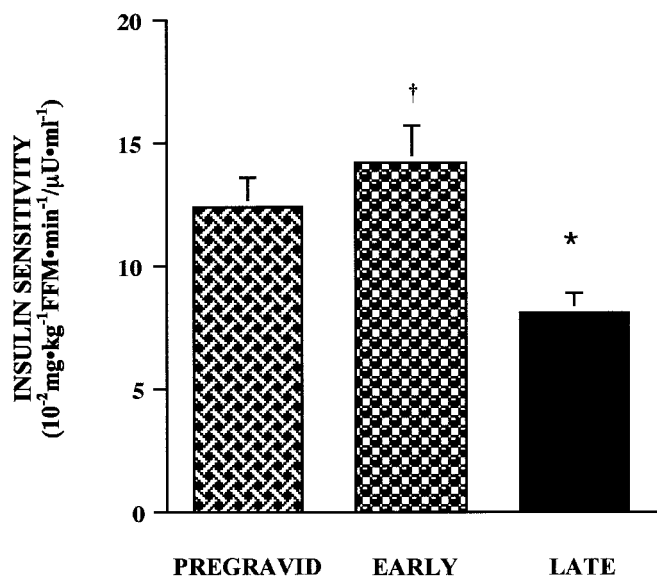


FIG. 1. Insulin sensitivity measured during euglycemic-hyperinsulinemic clamps performed pregravid and during early (12–14 weeks) and late (34–36 weeks) pregnancy. Data are means ± SE; *n* = 15. Glucose disposal rates and insulin concentrations were calculated for the final 150–180 min of the clamp. Units are expressed relative to FFM. *Significantly increased from pregravid, *P* < 0.02. †Significantly decreased from pregravid, *P* < 0.0001.

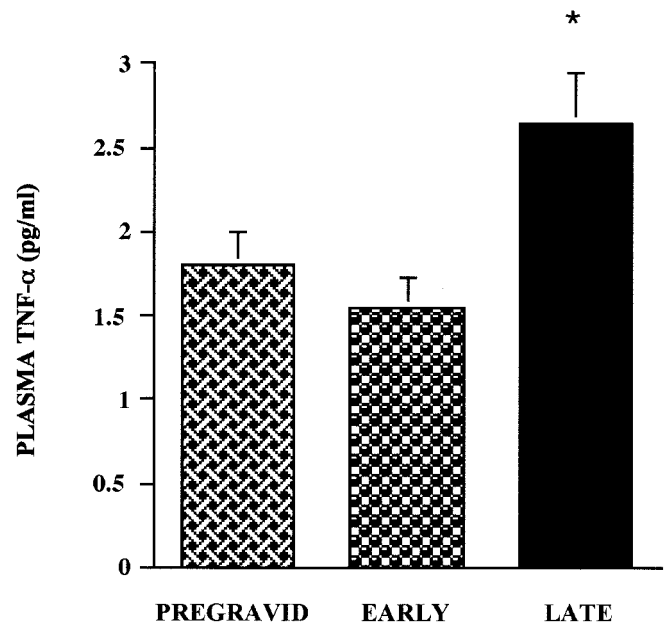


FIG. 2. Plasma TNF-α concentrations for all women pregravid and during early (12–14 weeks) and late (34–36 weeks) pregnancy. Data are mean ± SE; *n* = 15. Fasting blood samples were collected before each corresponding clamp. *Significantly higher than pregravid, *P* < 0.004.

51.6 pg · min⁻¹ · g⁻¹ placenta. Accumulation in the placental tissue during the course of perfusion was high (2,316 ± 1,671 pg/min) compared with fetal and maternal release (15.6 ± 4.9 pg/min and 493.2 ± 205.8 pg/min, respectively). Most of the TNF-α released in the medium (94 ± 3%) was delivered into the maternal circulation; 6 ± 3% was released into the fetal circulation.

In a univariate analysis, TNF-α was significantly correlated with insulin sensitivity pregravid (*r* = -0.54, *P* < 0.03) and during early (*r* = -0.68, *P* < 0.003) and late (*r* = -0.58, *P* < 0.02) pregnancy (Fig. 3). Plasma TNF-α data for one of the obese NGT subjects was >2 SDs from the mean; when these data were taken out of the analysis, the correlation between TNF-α and insulin sensitivity in late pregnancy improved to *r* = -0.69 and *P* < 0.006. Further,

TABLE 3

Longitudinal changes in TNF-α, leptin, and cortisol in women pregravid and during both early and late pregnancy

	Pregravid	Early pregnancy	Late pregnancy
TNF-α (pg/ml)			
Lean NGT	1.58 ± 0.32	1.28 ± 0.11	2.13 ± 0.11*
Obese NGT	2.05 ± 0.66	1.70 ± 0.65	2.80 ± 0.72
Obese GDM	1.75 ± 0.43	1.69 ± 0.29	2.84 ± 0.17†*
Leptin (ng/ml)			
Lean NGT	7.9 ± 1.4	8.9 ± 0.8	11.6 ± 1.6
Obese NGT	23.1 ± 9.4	39.2 ± 13.5	36.4 ± 12.0
Obese GDM	31.0 ± 8.2†	42.4 ± 7.0†	44.1 ± 12.2†
Cortisol (μg/dl)			
Lean NGT	13.8 ± 1.4	17.3 ± 1.9	32.3 ± 2.2*
Obese NGT	8.7 ± 1.2	15.5 ± 2.2*	30.0 ± 3.9*
Obese GDM	10.3 ± 1.6	17.0 ± 3.2*	32.1 ± 4.2*

Data are means ± SE; *n* = 5 per group. *Significantly different from pregravid, *P* < 0.01. †Significantly different from lean NGT group, *P* < 0.02.

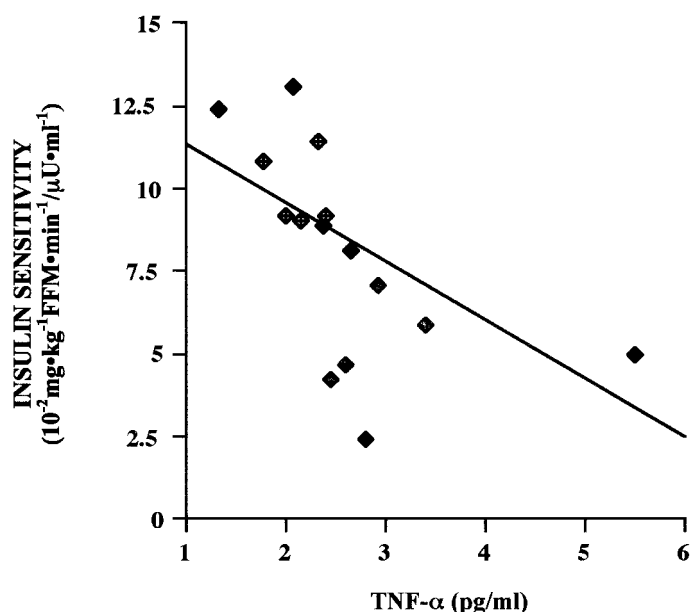


FIG. 3. Correlation between TNF- α and insulin sensitivity in late pregnancy ($r = -0.58$, $P < 0.02$). Data are shown for five lean women with NGT (\blacklozenge), five obese women with NGT (\blacklozenge), and five obese women with GDM (\blacklozenge). The correlation and regression equation based on data excluding one subject who was an outlier was $r = -0.69$, $P < 0.006$; $y = -4.242x + 18.426$ (see RESULTS).

the change in TNF- α from pregravid to late pregnancy was inversely related to the corresponding change in insulin sensitivity (Fig. 4). Using a stepwise regression analysis model, TNF- α was found to be a primary predictor of insulin sensitivity in pregnant women and explained >45% of the variance in the model (Table 4). Because plasma TNF- α was correlated with fat mass ($r = 0.68$, $P < 0.01$ in late pregnancy), the regression model was adjusted for body fat. After this adjustment, the strength of the corre-

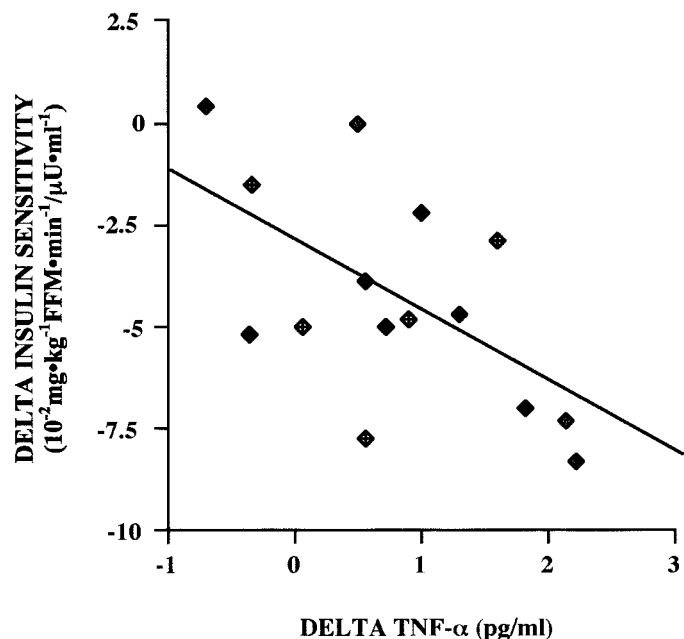


FIG. 4. Correlation between the change in TNF- α and the change in insulin sensitivity from pregravid to late pregnancy ($r = -0.60$, $P < 0.02$). Data are shown for five lean women with NGT (\blacklozenge), five obese women with NGT (\blacklozenge), and five obese women with GDM (\blacklozenge).

TABLE 4

Stepwise logistic regression: factors correlated with insulin sensitivity for the combined time periods

	r^2	Δr^2	P
Insulin sensitivity			
TNF- α (pg/ml)	0.453	—	0.0001
Leptin (ng/ml)	0.546	0.093	0.0001
Cortisol (μ g/dl)	0.616	0.070	0.0001
hCG (mIU/ml)	0.685	0.069	0.0001

lation was reduced, but TNF- α remained the best predictor of insulin sensitivity ($r = 0.46$, $P < 0.01$).

As expected, circulating leptin levels increased from pregravid (21.2 ± 4.9 ng/ml) to early pregnancy (31.5 ± 6.5 ng/ml) and remained elevated through late pregnancy (32.1 ± 6.8 ng/ml). Leptin levels were lower in lean women with NGT than in obese women with GDM (Table 3). In univariate analysis, there was an inverse correlation between leptin and insulin sensitivity ($r = -0.58$, $P < 0.01$), but when fat mass was entered as a covariate, the correlation was no longer significant ($r = 0.02$). In the stepwise regression analysis, leptin, unadjusted for fat mass, was the second best predictor of insulin sensitivity, contributing an additional 9% to the model (Table 4). Plasma cortisol also increased from pregravid (10.9 ± 1.0 μ g/dl) to early pregnancy (16.6 ± 1.3 μ g/dl) and increased further in late pregnancy (32.3 ± 2.2 μ g/dl). The correlation between cortisol and insulin sensitivity was significant ($r = -0.34$, $P < 0.05$). Cortisol entered the insulin sensitivity stepwise regression model at the third step and contributed an additional 7% to the variance.

Plasma estradiol, progesterone, and prolactin were all elevated during early pregnancy and increased further during late pregnancy (Table 5). Plasma HPL was significantly elevated and hCG was reduced in late compared with early pregnancy. Notably, there were no significant correlations between these hormones and insulin sensitivity in late pregnancy (hCG, $r = -0.29$, $P = 0.31$; HPL, $r = -0.24$, $P = 0.39$; prolactin, $r = -0.13$, $P = 0.67$; estradiol, $r = -0.12$, $P = 0.68$; and progesterone, $r = -0.10$, $P = 0.72$).

DISCUSSION

Historically, placental hormones are considered the primary mediators of insulin resistance during gestation (7–9). To our knowledge, however, there are no studies in

TABLE 5

Longitudinal change in reproductive hormones in women pregravid and during both early and late pregnancy

	Pregravid	Early pregnancy	Late pregnancy
hCG (IU/ml)	NA	42.4 ± 4.6	$19.4 \pm 4.0^*$
Estradiol (pg/ml)	132.6 ± 26.7	$1814.5 \pm 189.3^\dagger$	$11598.2 \pm 863.5^{*\dagger}$
Progesterone (ng/ml)	2.3 ± 0.9	$31.5 \pm 2.1^\dagger$	$137.5 \pm 14.2^{*\dagger}$
HPL (μ g/ml)	NA	0.9 ± 0.1	$7.8 \pm 0.5^*$
Prolactin (ng/ml)	10.6 ± 1.1	$28.8 \pm 1.8^\dagger$	$151.2 \pm 8.8^{*\dagger}$

Data are means \pm SE; $n = 15$. † Significantly different from pregravid, $P < 0.01$. $*$ Significantly different from early pregnancy, $P < .01$.

the literature that have directly examined the relationship between these hormones and insulin sensitivity throughout human pregnancy. Herein, we report for the first time that among women with NGT and GDM, TNF- α is a significant predictor of insulin resistance during pregnancy. Together with a small additive contribution from leptin and cortisol, TNF- α exerted a significant influence on insulin-mediated glucose disposal, whereas the contribution of HPL, hCG, estradiol, progesterone, and prolactin to insulin resistance was not significant. This observation challenges the long-held axiom that pregnancy-related insulin resistance is due to the production of placental reproductive hormones.

Subjects in the present study had a broad range of insulin sensitivity and body composition before pregnancy. Pregravid, 10 of the women had NGT and 5 had a high normal response; the latter 5 subsequently developed GDM during pregnancy. Differences in pregravid glucose metabolism were also evident in clamp-derived insulin sensitivity measures. Based on body composition measures, 5 of the 10 women with NGT were lean and 5 were obese, whereas all 5 of the women with GDM were obese. However, irrespective of these differences, changes in insulin sensitivity during pregnancy were similar for all women. Because the relative changes in insulin sensitivity were similar for lean and obese women with NGT and GDM, the data for the groups was combined to identify predictors of change in insulin sensitivity during pregnancy.

Circulating TNF- α showed a downward trend during early pregnancy and increased during the third trimester, thus mirroring insulin sensitivity changes during those periods. This observation is consistent with previous studies showing an increase in plasma TNF- α in late pregnancy (19,21) and demonstrates that when the same women are followed longitudinally, significant changes in TNF- α can be detected. Although reproductive hormones are increased 5- to 30-fold, they have relatively little predictive power, despite the fact that they have been traditionally associated with insulin resistance during pregnancy (7-9). Two other potential mediators of insulin resistance, leptin and cortisol, also correlated inversely with changes in insulin sensitivity in late pregnancy, but to a far lesser degree. Because TNF- α and leptin are secreted from fat cells as well as the placenta (13,14,23,34), we adjusted the data to account for changes in maternal fat accretion during the pregnancy period. When the data were analyzed with fat mass as a covariate, TNF- α remained a significant predictor of insulin sensitivity, whereas leptin was no longer significant. Thus, despite the increase in fat mass during gestation and the difference in body fat between lean women with NGT and obese women with NGT or GDM, plasma TNF- α was an independent correlate of insulin sensitivity during pregnancy.

The placenta is an important source of TNF- α in human pregnancy, with the greatest production rates evident in late gestation (12). We previously observed that, similar to leptin, increased TNF- α levels in pregnancy fall rapidly after delivery (35), consistent with the idea that the increase in circulating TNF- α during late pregnancy is due to placental secretion. Our *in vitro* model suggests that the vast majority of the TNF- α synthesized by the placenta is

delivered to the maternal side, with relatively little going into fetal circulation. Thus, TNF- α appears to be secreted asymmetrically into the maternal circulation in a manner similar to leptin (14). These findings may also help to explain the rapid reversal of insulin resistance after delivery, since maternal levels of TNF- α and leptin decrease substantially after delivery of the placenta.

Using an *in vitro* tissue explant incubation model, it has been shown that placentas from women with GDM release greater amounts of TNF- α in response to a glucose stimulus than those from women with NGT (36). Whether increased placental TNF- α production may explain increased insulin resistance in GDM compared with NGT is not clear; however, our data confirm a previous report that plasma TNF- α levels are higher in late gestation among women with GDM than in lean women with NGT (37). Because women with GDM were controlled with diet and had similar baseline glucose levels compared with obese pregnant women with NGT, the levels of TNF- α were not different. However, given that TNF- α may predict insulin resistance during late gestation, it could also contribute to greater insulin resistance in uncontrolled GDM subjects.

A number of studies have described a direct role for TNF- α in the pathophysiology of insulin resistance. *In vitro* studies have shown that TNF- α downregulates insulin receptor signaling in cultured adipocytes (38), hepatocytes (39), and skeletal muscle (40). Furthermore, increased TNF- α is associated with insulin resistance in a broad range of conditions including obesity (16), aging (17), sepsis (18), and after muscle damage (15). TNF- α activates a pathway that increases sphingomyelinase and ceramides and appears to interfere with insulin receptor autophosphorylation. Recently, it has been shown that TNF- α promotes serine phosphorylation of insulin receptor substrate (IRS)-1, thus impairing its association with the insulin receptor (41). In pregnancy, there is evidence that insulin receptor and IRS-1 tyrosine phosphorylation are impaired, and serine phosphorylation is increased in late gestation in skeletal muscle (42,43). Therefore, it seems plausible that elevated levels of TNF- α in late gestation could attenuate insulin signaling, thus causing the decreased insulin sensitivity observed in pregnancy. Preliminary data from our laboratory suggest that insulin receptor and IRS-1 changes in skeletal muscle are reversible after pregnancy, indicating that TNF- α may be an important hormonal mediator responsible for insulin resistance in human pregnancy (J.P.K., P.M.C., unpublished observations).

Maternal circulating leptin increases during pregnancy, with most of the increase occurring in the first trimester (26). Whereas the placenta appears to be a primary site of maternal leptin production (14), secretion from the fat cell is also important, and plasma leptin is positively correlated with level of obesity (44). In the present study, leptin was increased in all women in early pregnancy, remained elevated in late pregnancy, and was highest in the more obese GDM group. To adjust for the possible confounding effect of obesity and increased fat mass on the relationship between leptin and insulin sensitivity, we covaried for body fat and found that the correlation was no longer significant. Because the increased leptin *per se* was not predictive of insulin sensitivity, one interpretation is that

in addition to insulin resistance, leptin resistance may also develop in late pregnancy.

Data from the present study indicate that reproductive hormones and cortisol do not significantly correlate with the change in insulin sensitivity during pregnancy. Because these hormones have established diabetogenic properties, possibly acting through lipolytic mechanisms (8,45), one would expect to see some association with the insulin resistance that was present at this time. These data suggest that whereas the placental hormones and cortisol have specific functions in the mother and feto-placental unit, their association with maternal insulin sensitivity is limited (placental growth hormone notwithstanding). However, this does not preclude the possibility that these hormones can play a permissive role in insulin resistance in pregnancy by potentiating the effects of more direct mediators such as TNF- α . Alternatively, these hormones could have an effect on other non-placental-derived mediators of insulin sensitivity such as free fatty acids, resistin, or adiponectin.

In conclusion, insulin resistance during late gestation is significantly correlated with changes in circulating TNF- α , irrespective of fat mass. The primary source of the increased TNF- α appears to be the placenta. Studies in vitro have shown that TNF- α inhibits insulin signaling and insulin-regulated glucose uptake, thus suggesting that the insulin resistance of pregnancy may be mediated through this cytokine. These observations provide an alternative to the placental-derived reproductive hormone paradigm to explain insulin resistance during pregnancy. The regulation of TNF- α may provide an important target for physiological interventions designed to reduce the risk of adverse pregnancy outcomes related to insulin resistance.

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