Is There Any Link Between Insulin Resistance and Inflammation in Established Preeclampsia?

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Both insulin resistance and inflammation may contribute to the onset of preeclampsia. They also could be interrelated. We studied the relationship between inflammatory cytokines and markers of insulin resistance. During their third trimester, 22 proteinuric preeclamptic women and 16 normotensive controls underwent intravenous glucose tolerance test (minimal model). Preeclamptic women were more insulin-resistant ($P = .009$), and they had higher levels of serum soluble tumor necrosis α receptor II (TNFαRII) ($P = .002$), triglycerides ($P = .006$), uric acid ($P = .001$), and leptin ($P = .002$) than did the controls. However, the study groups did not differ in serum TNFα, C-reactive protein (CRP), interleukin-6 (IL-6), sex hormone-binding globulin (SHBG), and high-density lipoprotein-2 (HDL_2)-cholesterol. In multiple regression analysis only SHBG ($P = .01$) and triglycerides ($P = .0036$) were associated with insulin sensitivity independently of body mass index (BMI), weight gain, HDL_2-cholesterol, CRP, TNFα, and TNFαRII, IL-6, and leptin. We conclude that insulin resistance and the inflammatory markers studied were not associated in established preeclampsia.

BOTH INSULIN RESISTANCE and inflammation have been reported to contribute to the onset of hypertension and coronary artery disease[1] and they may be present in hypertensive disorders of pregnancy.[2-4] Insulin resistance, which is present also in milder form in late normal pregnancy, has long been ascribed to rises in cortisol and in placental-derived hormones including human placental lactogen, progesterone, and estrogen.[5] The causes for its further enhancement in hypertensive pregnancies are unknown.[6] One explanation could be inflammation, and indeed rises in some inflammatory markers predict the onset of insulin resistance in pregnant subjects.[7,8] Among these candidates, tumor necrosis factor α (TNFα) and leptin are known to be produced, besides adipose tissue, also in the placenta and could therefore play a central role in insulin resistance during pregnancy.[8] The current study aimed to determine whether insulin resistance with a number of its markers (leptin, uric acid, lipids, lipoproteins, sex hormone-binding globulin [SHBG]) is related to a number of inflammatory cytokines (TNFα and its soluble receptor II ([TNFαRII], interleukin-6 [IL-6], and C-reactive protein [CRP]) in established preeclampsia.

MATERIALS AND METHODS

Sample

Power calculations showed that 16 patients with preeclampsia and 16 controls were needed to detect a 33% difference in mean plasma THFαRII level between the 2 groups with 80% power at P level of .05. With the permission of our Ethics Committee, and after receiving the informed consent of the volunteers, we studied 38 nulliparous women between 29 and 39 weeks of gestation (Table 1). The patients were women with established preeclampsia and the controls were 16 healthy nulliparous women with singleton pregnancy, who had a similar body mass index (BMI) and were studied at the same time and at the same gestational weeks. None of the control women developed hypertension or proteinuria during the remainder of their pregnancies.

Preeclampsia was defined as blood pressure greater than 140/90 mm Hg measured at least 2 times 6 hours apart and proteinuria $\geq$0.3g/d after 20 weeks of gestation. To exclude the presence of glucose intolerance, all subjects underwent a 2-hour oral glucose tolerance test (OGTT, 75 g) after an overnight fast at 8 AM 1 to 7 days earlier. Only women whose glucose tolerance was normal (fasting $\leq$4.5 mmol/L; 1 hour $\leq$9.1 mmol/L; 2 hour $\leq$7.9 mmol/L) were accepted for the study.

Measures

One to 7 days after the OGTT the whole-body insulin sensitivity of the subjects was measured using the minimal model technique after an overnight fast and in the absence of any medication as previously reported.[4] Briefly, a bolus of glucose (0.3 g/kg body weight) was injected intravenously (IV) at 9 AM, followed by a dose of insulin (0.03 IU/kg IV; Velosulin Human, Novo Nordisk Pharmaceuticals, Bagsværd, Denmark) 20 minutes later. Frequent blood samples were collected for up to 3 hours; the insulin sensitivity was calculated from the concomitant changes in glucose and insulin.[4]

Serum samples collected before the injection of glucose were assayed for markers reflecting insulin senstivity, such as serum leptin, triglyceride, high-density liporpotein (HDL)-cholesterol, and urate levels. Serum leptin levels were assayed by specific radioimmunoassay (RIA; Human leptin RIA kit, Linco Research, St Louis, MO), and all samples were run in the same batch of the assay to minimize the variation. The intra-assay coefficient of variation was at low concentration (2.8 μg/L) 4.7%, and at medium concentration (15.6 μg/L) 3.8%. The measurements of insulin and insulin sensitivity have been described previously.[4] Serum triglyceride concentrations were analyzed in a Technicon AutoAnalyzer II (Technicon Instruments, Tarrytown, NY). For HDL_2-cholesterol analysis, HDL was separated from serum by precipitation of the other lipoproteins with heparin-manganese chloride. The supernatant was further fractionated into HDL_2 and HDL3 by precipitation of HDL3 with 0.11% dextran sulfate. Serum uric acid was measured with an enzymatic colorimetric test (Boehringer, Mannheim, Germany).

The same sera were used for the assessment of inflammation markers such as TNFα, soluble TNFα-RII, IL-6 and sensitive CRP. All cytokines were measured with commercially available sandwich-type en-

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Table 1. Clinical Characteristics of the Study Population

Characteristic	Women With Preeclampsia (n = 22)	Normotensive Pregnant Women (n = 16)	*P* Value
Age (yr)	30.4 ± 1.0	31.8 ± 1.1	NS
Week of gestation at study	36.3 ± 0.5	35.2 ± 0.7	NS
Pre-pregnancy BMI (kg/m^2)	22.6 ± 0.5	21.9 ± 0.8	NS
BMI at study (kg/m^2)	27.0 ± 0.7	26.5 ± 0.8	NS
Proteinuria (g/24 h)	2.0 ± 0.6		
Uric acid (mmol/L)	0.37 ± 0.02	0.27 ± 0.01	.0001
Fasting insulin (μU/mL)	7.4 ± 0.6	6.1 ± 0.6	NS
Systolic blood pressure (mm Hg)	142 ± 2	120 ± 3	.0001
Diastolic blood pressure (mm Hg)	96 ± 2	73 ± 2	.0001
Weeks of gestation at delivery	38.3 ± 0.4	40.4 ± 0.3	.0003
Infant's birth weight (g)	2,764 ± 120	3,546 ± 89	.0001
Placental weight (g)	552 ± 30	648 ± 25	.03

NOTE. Data are means ± SE.
Abbreviation: NS, not significant.

zyme-linked immunoassays using serum samples. IL-6 and TNFα were measured with Diaclone kits (Besancon, France) with sensitivity of 0.8 ng/L (reference values, <3.5 ng/L for IL-6 and 10 ng/L for TNFα). Soluble TNR receptor type II levels were measured using a sandwich-type enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN). The sensitivity of the assay is 10 ng/L and the normal range is 1,500 to 2,500 ng/L.

Statistical Analysis

We compiled all data on a database, which was analyzed by using NCSS 2000 (NCSS, Kaysville, UT). Continuous variables are presented as the mean ± SEM. Leptin values were logarithmically transformed to normalize the distribution. Paired or where appropriate unpaired Student's *t* tests were used for comparisons. Multiple regression analysis was used with insulin sensitivity as the dependent variable and BMI, weight gain, serum triglycerides, serum HDL_2-cholesterol, CRP, TNFα-RII, serum IL-6, SHBG, and serum leptin as independent variables.

RESULTS

Preeclamptic women had higher levels of uric acid and triglyceride, and blood pressure was also higher than in normotensive women (Table 1). Preeclamptic women had lower insulin sensitivity and higher leptin levels than normotensive women (Table 2). Of the inflammatory markers, only TNFαRII was elevated in preeclamptic women compared to controls, but the elevation in TNFα did not reach significance (Table 2).

In multiple regression analysis, only triglycerides and SHBG emerged as independent mediators of insulin resistance, while none of the inflammation markers showed a significant trend (Table 3). These risk factors explained 61% of enhanced insulin resistance in preeclampsia.

DISCUSSION

In addition to being more insulin-resistant, preeclamptic women had elevated serum TNFαRII, triglyceride, uric acid, and leptin levels. However, only triglycerides ($P = .0036$) were associated independently of BMI, weight gain, HDL_2-cholesterol, CRP, TNFα, IL-6, and leptin. Thus, these data do not suggest a significant role for the inflammatory factors investigated in the development of insulin resistance in established preeclampsia.

There are increasing data supporting the role of insulin resistance in preeclampsia,[4,6] although this evidence has not been seen in all studies.[9] The etiologic role of insulin resistance for the onset of preeclampsia gains support from data showing that insulin resistance in the first trimester, associated with low SHBG, predicts development of preeclampsia.[2] Indeed, low

Table 2. Biochemical Characteristics of the Study Population

Characteristic	Women With Preeclampsia (n = 22)	Normotensive Pregnant Women (n = 16)	*P* Value
Insulin sensitivity ($10^{-4} \cdot min^{-1}/\mu$U/mL)	1.1 (0.7)	1.8 (0.8)	.009
Serum leptin (μg/L)	34.6 (3.9)	20.0 (3.3)	.002
SHBG (nmol/L)	444.3 (99.4)	430 (95.0)	.7
Serum triglyceride (mmol/L)	3.6 (0.9)	2.3 (0.8)	.006
Serum HDL_2 cholesterol (mmol/L)	0.3 (0.2)	0.4 (0.2)	.1
Serum TNFα (ng/L)	17.7 (7.1)	12.4 (4.2)	.09
Serum TNFα-RII (ng/L)	2,415 (644)	1,824 (581)	.002
Serum CRP (mg/L)	4.7 (4.2)	3.6 (2.8)	.7
Serum IL-6 (ng/L)	4.2 (3.9)	3.1 (2.7)	.7

NOTE. Data are as means (±SD).

Table 3. Contribution of Different Risk Factors to the Insulin Sensitivity in Whole Population (multiple regression analysis)

Risk Factor	Univariate Analysis *P*	R^2	Multivariate Analysis *P*
BMI	.018	.15	.15
Weight gain	.069	.089	.059
Triglycerides	.002	.23	.0035
HDL_2-cholesterol	.84	.0011	.22
CRP	.13	.062	.94
TNFα-RII	.056	.099	.08
IL-6	.16	.055	.34
SHBG	.33	.027	.013
Leptin (μg/L)	.039	.12	.86

NOTE. R^2 after multiple regression: 0.61.

levels of SHBG appear to be a relatively good marker of hyperinsulinemia and insulin resistance in nonpregnant subjects[10,11] and may predict the onset of type 2 diabetes.[12] In our study, SHBG showed no significant difference between preeclamptic and normotensive women, although in multivariate analysis, SHBG emerged as a significant factor in insulin resistance.

Of the inflammatory cytokines, only soluble TNFαRII was elevated in preeclampsia, with a nonsignificant trend of TNFα in the same direction. TNFα is involved in the immune response and is also a possible link between obesity, impaired glucose tolerance, and type 2 diabetes.[13]. Plasma levels of soluble TNFαRII, reflecting the action of TNFα at tissue level, is a good marker of TNFα-induced insulin resistance, as it is elevated both in adipose tissue and plasma.[14,15] It was recently reported that TNFα is one of the primary mediators of insulin resistance in normal pregnancy[8] and our data on high TNFαRII levels in preeclamptic women seem to support this claim. However, the association between TNFαRII and insulin resistance did not reach statistical significance, and this seems to argue against the role of these inflammation markers in insulin resistance in established preeclampsia. One possible explanation for the lack of association may be the placental production of TNFα.[8] It is possible that during pregnancy placental TNFα production may confound any association between insulin sensitivity and TNFα (reflected by soluble TNFαRII) concentration.

Contradictory findings have been reported concerning the role of CRP, another inflammatory marker, as a predictor of preeclampsia.[16,17] In established preeclampsia, CRP has been reported to be higher than in normotensive controls, although this association was lost after adjustment for maternal weight.[18] We did not find any difference in CRP levels between preeclamptic and control women, which could be due to matching the control group by BMI, a factor known to influence both parameters.[18,19] Although CRP tended to be associated with insulin resistance in univariate analysis ($P = .06$), this trend vanished in multivariate analysis ($P = .9$).

In conclusion, our results did not show correlations between insulin resistance and some markers of inflammation in estableshed preeclampsia.

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