Evidence of a State of Increased Insulin Resistance in Preeclampsia

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Similarities in certain biochemical variables between preeclampsia and the insulin resistance syndrome imply a possible link between insulin resistance and preeclampsia. We measured insulin sensitivity by the minimal model technique between 29 and 39 weeks of gestation in 22 preeclamptic and 16 control women, whose glucose tolerance was first confirmed as normal by an oral glucose tolerance test. In addition, we measured the fasting levels of serum C-peptide, uric acid, lipids, and lipoproteins. Preeclamptic women showed a higher insulin response ($P = .001$) during the oral glucose tolerance test than the controls. Insulin sensitivity in preeclamptic women ($1.11 \pm 0.15 \times 10^{-4} \cdot min^{-1} \cdot \mu U/mL$) was 37% lower ($P = .009$) than in control women ($1.77 \pm 0.19 \times 10^{-4} \cdot min^{-1} \cdot \mu U/mL$). The free fatty acid (FFA) concentration in preeclamptic women (0.17 ± 0.01 g/L, $P = .0004$) was 70% higher than in control women (0.10 ± 0.01 g/L). Also, baseline serum levels of C-peptide, uric acid, and triglyceride were higher in preeclamptic women. Insulin sensitivity increased fourfold to fivefold within the first 3 postpartum months, but insulin sensitivity in preeclamptic women was still 26% lower ($P = .04$) than in control women. Preeclampsia is a state of increased insulin resistance, and it persists for at least 3 months after pregnancy. This may be a pathogenetic factor in preeclampsia and may contribute to the excess cardiovascular morbidity among women with prior preeclampsia.

PREECLAMPSIA, which affects 2% to 6% of first pregnancies, remains the most common cause of maternal and neonatal death.[1] Preeclampsia manifests as increased blood pressure (BP), proteinuria, generalized vasoconstriction, and increased platelet consumption,[2] and is therefore often regarded as a pregnancy-specific vascular disorder. However, in their subsequent life, women with prior preeclamptic pregnancies have shown an increased risk of cardiovascular disorders.[3,4] This may imply the existence of some inherent biochemical abnormality, which is manifest during pregnancy as preeclampsia and later as an increased risk for cardiovascular disease.[5]

The insulin resistance syndrome, which consists of reduced glucose utilization, hyperinsulinemia, hypertriglyceridemia, decreased high-density lipoprotein (HDL) cholesterol, hypertension, and hyperuricemia, predisposes men and nonpregnant women to an increased risk of cardiovascular disorders.[6] In preeclamptic pregnancies, metabolic changes similar to those in the insulin resistance syndrome are also present,[7,8] and in addition, hyperinsulinemia[9] and hyperandrogenism[10] persist for up to 17 years after preeclamptic pregnancy. Although this may indicate a link between preeclampsia and the insulin resistance syndrome, no data are available indicating true insulin sensitivity in preeclampsia. Therefore, we compared insulin sensitivity as assessed by an intravenous glucose tolerance test between preeclamptic and normotensive pregnancies.

SUBJECTS AND METHODS

With the permission of the ethics committee, we recruited 22 nulliparous, previously healthy preeclamptic women from the antenatal ward of Helsinki University Hospital between January 1, 1996 and April 30, 1997 (Table 1). Preeclampsia was defined as BP greater than 140/90 mm Hg confirmed by two measurements (in the sitting position) at least 6 hours apart and proteinuria greater than 0.3 g/24-hour urine collection between 29 and 39 weeks of gestation. The prepregnancy body mass index was 18.3 to 29.1 kg/m^2, all preeclamptic women were carrying a single fetus, and none used antihypertensive medication, aspirin, or corticosteroids. Excluded were patients with preeclampsia onset before 28 weeks of gestation and those with BP greater than 170/110 mm Hg and/or proteinuria greater than 5 g/24 h (or dipstick +++), since these patients often need immediate medication. In the same period, we also recruited 16 healthy nulliparous pregnant controls with singleton pregnancies who had a similar prepregnancy body mass index as the preeclamptic patients. The controls were studied at the similar weeks of gestation. None developed hypertension during the remaining weeks of gestation. All study subjects provided informed consent. The two study groups were comparable with respect to age, race, and body mass index before pregnancy, weight gain during pregnancy, and gestational age during the study; however, preeclamptic women gave birth earlier and had lighter infants than the control women (Table 1). Only the preeclamptic group consisted of hospitalized patients, but these women were not on bed rest. The study subjects were invited to the reexamination an average of 12 weeks (range, 6 to 22) after delivery, and 14 women in the preeclamptic group and 11 women in the control group complied with this request. In women with prior preeclampsia, the proteinuria disappeared and BP was reduced, although systolic and diastolic BP in the preeclamptic group were still significantly higher than in the controls (Table 1). All women were amenorrheic at the follow-up examination and did not use antihypertensive medication or hormonal contraceptives.

Protocol

To exclude the presence of glucose intolerance, all subjects underwent a 2-hour oral glucose tolerance test (75 g glucose) after an overnight fast at 8 AM 1 to 7 days earlier. Only women whose glucose tolerance was normal (fasting, ≤4.5 mmol/L; 1 hour, ≤9.1 mmol/L; 2 hours, ≤7.9 mmol/L) were accepted for the intravenous glucose tolerance test. In view of the interactions between insulin and the release of free fatty acids (FFAs) from adipose tissue,[11] we also assessed serum levels of insulin and total FFAs during the oral glucose tolerance test. We assessed insulin sensitivity by an intravenous glucose tolerance test using the minimal model developed by Bergman.[12] A bolus of glucose (0.3 g/kg body weight) was injected intravenously at 9 AM after an overnight fast, followed by a bolus of human regular insulin (Velosulin Human; Novo Nordisk Pharmaceuticals, Bagsvaerd, Denmark; 0.03 IU/kg) 20 minutes later. Blood samples were collected as follows: two

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Submitted October 7, 1998; accepted January 25, 1999.

Supported by grants from the Finnish Academy of Science, the Paavo Nurmi Foundation, and the Helsinki University Central Hospital.

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0026-0495/99/4807-0015$10.00/0

Table 1. Clinical Characteristics of the Study Population

Characteristic	Women With Preeclampsia (n = 22)	Normotensive Pregnant Women (n = 16)
Age (yr)	30.4 ± 1.0	31.8 ± 1.1
Race	Caucasian	Caucasian
Prepregnancy body mass index (kg/m²)	22.6 ± 0.5	21.9 ± 0.8
Weeks of gestation at study	36.3 ± 0.5	35.2 ± 0.7
Body mass index at study (kg/m²)	27.0 ± 0.7	26.5 ± 0.8
Weight gain during pregnancy up to time of study (kg)	12.8 ± 1.0	12.5 ± 0.8
Systolic BP (mm Hg)	142 ± 2*	120 ± 3
Diastolic BP (mm Hg)	96 ± 2*	73 ± 2
Weeks of gestation at delivery	38.3 ± 0.4†	40.4 ± 0.3
Infant birth weight (g)	2,764 ± 120*	3,546 ± 89
Women reexamined after delivery (n)	14	11
Weeks after delivery at reexamination	12.8 ± 1.0	13.6 ± 1.0
Body mass index at reexamination (kg/m²)	23.9 ± 0.8	23.5 ± 1.0
Systolic BP at reexamination (mm Hg)	115 ± 2§	107 ± 2
Diastolic BP at reexamination (mm Hg)	75 ± 2‡	66 ± 2

NOTE. Data are the mean ± SE.

*P = .0001, †P = .0003, ‡P = .002, §P = .02: v normotensive pregnant women.

at baseline and 4, 6, 8, 10, 19, 22, 29, 37, 67, 90, and 180 minutes after administration of the glucose bolus. Insulin sensitivity was evaluated from the disappearance curves for glucose and insulin by the minimal model computer program. This model accurately reflects true insulin sensitivity, as confirmed and validated by use of the hyperinsulinemic-euglycemic clamp technique.[12] Moreover, during pregnancy, values for insulin sensitivity evaluated by the hyperinsulinemic-euglycemic clamp or the minimal model are in good correlation (r = .58).[13] The first-phase insulin response, an indicator of pancreatic β-cell function in the first 10 minutes during intravenous testing, was calculated separately. Baseline blood samples were also assessed for C-peptide, uric acid, lipid, and lipoprotein levels. Insulin sensitivity was examined by the same technique in 14 preeclamptic women and 11 control women an average of 12 weeks (range, 6 to 22) after delivery (Table 1).

Laboratory Methods

Blood glucose levels were measured with the enzymatic-amperometric method (ESAT 6660 analyzer; Eppendorf-Nelheler-Hinz, Hamburg, Germany). The coefficient of variation in between-run analyses was 2.6%. Sera for C-peptide and insulin assessment were stored frozen (−20°C) until assayed in the same batch by radioimmunoassay ([RIA] C-peptide: RIA-coat C-Peptide; Byk-Sangtek Diagnostica, Dietzenbach, Germany; and insulin: Phadeseph Insulin RIA kit; Kabi, Pharmacia, Uppsala, Sweden). The intraassay coefficient of variation was 3.6% for C-peptide and 7.7% for insulin. Serum uric acid, triglyceride, and total and lipoprotein cholesterol levels were measured as previously described.[9] The intraassay and interassay coefficients of variation were 1.2% and 2.2% for uric acid, less than 0.5% and 2.0% for triglycerides and total cholesterol, 4.4% and 5.4% for HDL cholesterol, less than 4.0% and less than 6.0% for HDL_2 cholesterol, and 3.0% and 6.8% for HDL_3 cholesterol. FFAs were extracted with the method of Folch et al.[14]

Statistics

Data analyses were performed with the Statview II program (Abacus Concepts, Berkeley, CA). Continuous variables are presented as the mean ± SE. The results were tested for normality. Comparisons were performed between groups with Student's two-tailed unpaired t test and within each group with Student's two-tailed paired t test. Relations between insulin sensitivity and different variables were investigated by linear regression analysis. The areas under the response curves for blood glucose and serum insulin were calculated by the trapezoidal rule. A disposition index reflecting β-cell function in relation to insulin sensitivity was calculated as insulin secretion (expressed as incremental

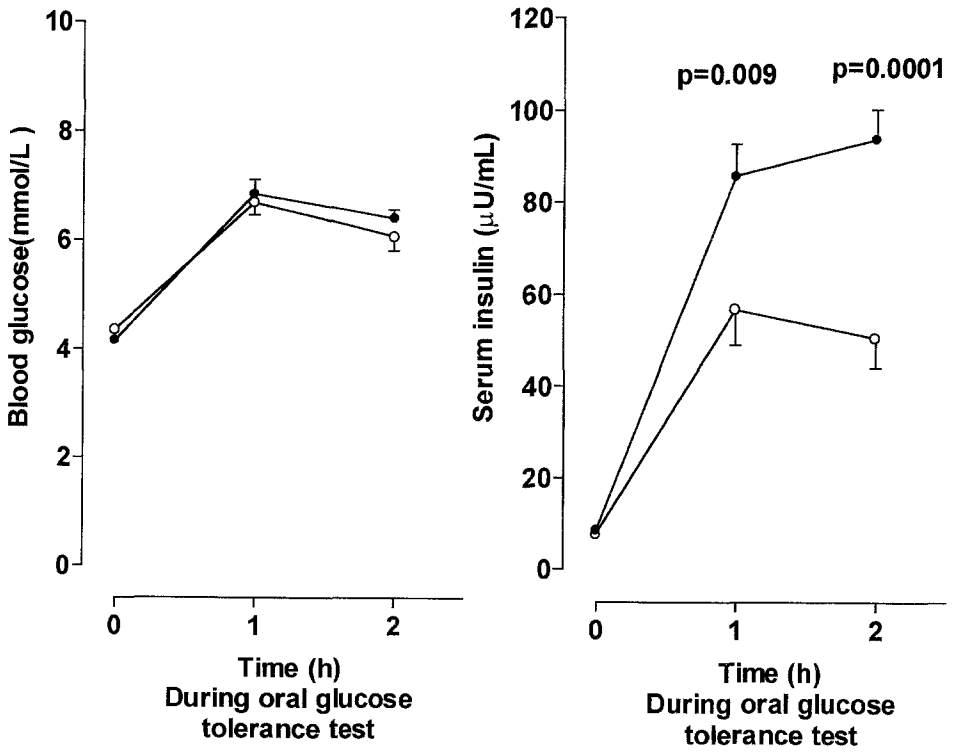

Fig 1. Blood glucose and serum insulin before and after a standard oral glucose dose (75 g) in preeclamptic women (●) and normotensive pregnant control women (○). Data are the mean ± SE.

area under the insulin curve during the first 10 minutes) multiplied by insulin sensitivity.

RESULTS

Preeclamptic and control women had a similar blood glucose response in the oral glucose tolerance test, but the insulin response in preeclamptic women was significantly higher (Fig 1). The area under the insulin curve in preeclamptic women (8,221 ± 560 μU/mL · min) was 59% larger ($P = .001$) than in the control women (5,157 ± 611 μU/mL · min). In contrast to the normal basal insulin levels, fasting C-peptide levels in preeclamptic women (0.73 ± 0.04 nmol/L) were higher ($P = .03$) than in control women (0.58 ± 0.05 nmol/L). The C-peptide to insulin ratio showed no difference between the two groups.

The range of insulin sensitivity was $0.26 \times 10^{-4} \cdot min^{-1} \cdot$ μU/mL to $2.52 \times 10^{-4} \cdot min^{-1} \cdot$ μU/mL in preeclamptic women and $0.45 \times 10^{-4} \cdot min^{-1} \cdot$ μU/mL to $3.62 \times 10^{-4} \cdot min^{-1} \cdot$ μU/mL in controls. As a mean value, insulin sensitivity was 37% lower ($P = .009$) in preeclamptic women versus the controls (Fig 2). The first 10-minute insulin response, expressed as the area under the curve, was 53% higher in preeclamptic women (971.0 ± 81.6 μU/mL · min) than in control women (633.8 ± 61.5 μU/mL · min, $P = .004$). The disposition index did not differ between preeclamptic women and controls (0.086 ± 0.008 *v* 0.098 ± 0.010, $P = .48$).

Twelve weeks after delivery, insulin sensitivity was increased 4.6-fold in preeclamptic women (from 1.11 ± 0.15 to $5.10 \pm 0.37 \times 10^{-4} \cdot min^{-1} \cdot$ μU/mL, $P = .0001$) and 3.8-fold in controls (from 1.77 ± 0.19 to $6.86 \pm 0.79 \times 10^{-4} \cdot min^{-1} \cdot$ μU/mL, $P = .0001$). Yet postpartum insulin sensitivity was reduced 26% in the preeclamptic group compared with the control group ($P = .04$) (Fig 2). The disposition index did not differ between women with prior preeclampsia and controls (0.168 ± 0.025 *v* 0.152 ± 0.016, $P = .62$). No significant correlations emerged between insulin sensitivity during pregnancy and after delivery in the two groups.

Insulin sensitivity correlated negatively with the weeks of gestation in preeclamptic women ($r = -.53$, $P = .01$), but not in controls ($r = -.13$, $P = .64$). The insulin response to oral glucose and insulin sensitivity in the intravenous glucose tolerance test showed a strong negative correlation in preeclamptic women ($r = -.70$, $P = .0004$), but not in control women ($r = -.45$; $P = .10$). Furthermore, insulin sensitivity correlated negatively with baseline C-peptide concentrations both in preeclamptic women ($r = -.62$; $P = .002$) and in control women ($r = -.58$, $P = .02$) (Fig 3).

Serum uric acid levels were higher in preeclamptic women (0.37 ± 0.02 mmol/L, $P = .0001$) than in control women (0.27 ± 0.01 mmol/L). In the whole study population, insulin sensitivity was negatively related to the uric acid level ($r = -.35$; $P = .03$).

In preeclamptic women, serum triglycerides were 37% higher ($P = .004$) than in control women (3.15 ± 0.19 *v* 2.29 ± 0.21 mmol/L), but between the two groups, total and lipoprotein cholesterol levels did not differ (data not shown). HDL_2 cholesterol tended to be lower in preeclamptic women (0.33 ± 0.04 mmol/L, $P = .09$) than in control women (0.44 ± 0.06 mmol/L). Baseline serum FFA concentrations

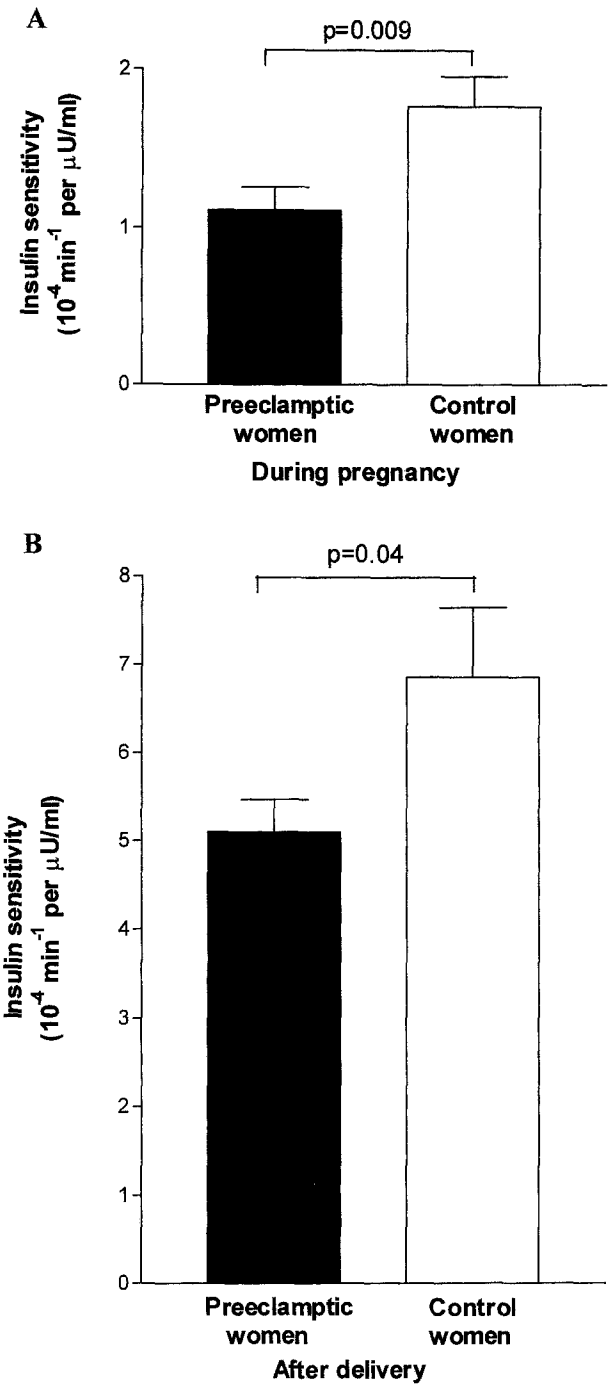

Fig 2. Insulin sensitivity in intravenous glucose tolerance assessed by minimal model analysis in women with preeclampsia and normotensive control women (A) during pregnancy and (B) 12 weeks after delivery. Data are the mean ± SE.

were 70% higher in preeclamptic women (0.17 ± 0.01 g/L) than in control women (0.10 ± 0.01 g/L, $P = .0004$), but this difference disappeared 2 hours after oral intake of glucose (Fig 4). The oral intake of glucose was accompanied by a 38% reduction in FFAs in preeclamptic women ($P = .0001$) but only an 18% reduction in control women ($P = .32$) (Fig 4). In the whole study population, insulin sensitivity was negatively

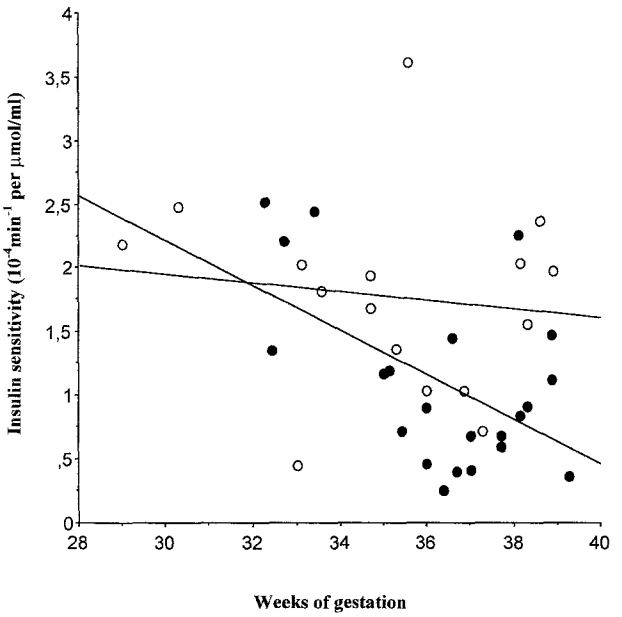

Fig 3. Correlation between insulin sensitivity and weeks of gestation in preeclamptic women (●), $r = -.53$, $P = .01$, and normotensive pregnant control women (○), $r = -.13$, $P = .64$.

related to triglycerides ($r = -.48$; $P = .002$) but not to FFAs or total or lipoprotein cholesterol. In the whole study population, the area under the insulin curve during the oral glucose tolerance test was positively related to the reduction in FFAs ($r = .43$, $P = .015$).

DISCUSSION

Changes in endothelial function and vasoactive agents have been proposed as possible pathogenetic mechanisms of preeclampsia.[1] However, an open question is whether these changes are primary or merely reflect some inherent biochemical abnormality in these women. Our group[7] and others[8] have reported that the high triglyceride, low HDL_2 cholesterol, and other metabolic characteristics observed in preeclampsia resemble those found in the insulin resistance syndrome. Because no data existed on insulin sensitivity in women with preeclampsia, we measured it with the minimal model method, validated for use also during and after pregnancy.[12,13] In addition, because we wished to exclude the possibility of glucose intolerance, which is known to be associated with insulin resistance, we first confirmed normal glucose tolerance in our study subjects.

Insulin sensitivity, as assessed by the euglycemic-hyperinsulinemic clamp technique or the intravenous glucose tolerance test by minimal model analysis, is lower during normotensive pregnancy compared with the nonpregnant state.[15,16] The cause of this phenomenon is unknown, but several pregnancy-associated hormones such as human placental lactogen, cortisol, and prolactin may be involved.[16] Hyperinsulinemia and a higher insulin to glucose ratio have been previously reported in association with hypertension in pregnancy and/or preeclampsia.[17-20] We now report that insulin sensitivity is lower in clear-cut pure preeclampsia versus normotensive pregnancy. This reduction was not limited to pregnancy, but persisted for as long as 3 months after delivery. Moreover, insulin sensitivity became lower with an increasing insulin response during the oral glucose tolerance test. Evidently, pancreatic β-cell function in preeclamptic women was normal, as observed from the similar disposition index during pregnancy. This could explain the presence of normoglycemia in these women despite significantly lower insulin sensitivity. The negative correlation between insulin sensitivity and weeks of gestation emerged only in preeclamptic women, indicating in these patients a further decrease in insulin sensitivity during the third trimester.

Our finding of greater insulin resistance in the postpartum period in Caucasian preeclamptic women is in agreement with previous data for Chinese women[21] obtained 2 months postpartum by the insulin suppression test, but is in disagreement with data for African-American women[22] obtained 3 to 6 months postpartum by the hyperinsulinemic-euglycemic clamp technique. These discrepancies might be explained by differences in the method of measuring insulin sensitivity or in the interval between delivery and each study. A difference in race can also be a confounding factor.

Our data do not allow us to deduce whether the increased insulin resistance in preeclamptic women is a primary change and therefore an etiological factor, or whether it is secondary to sympathetic overactivity,[23] decreased muscular blood flow,[1] or increased lipolysis.[24] We can only speculate on the mechanisms by which increased insulin resistance could contribute to the

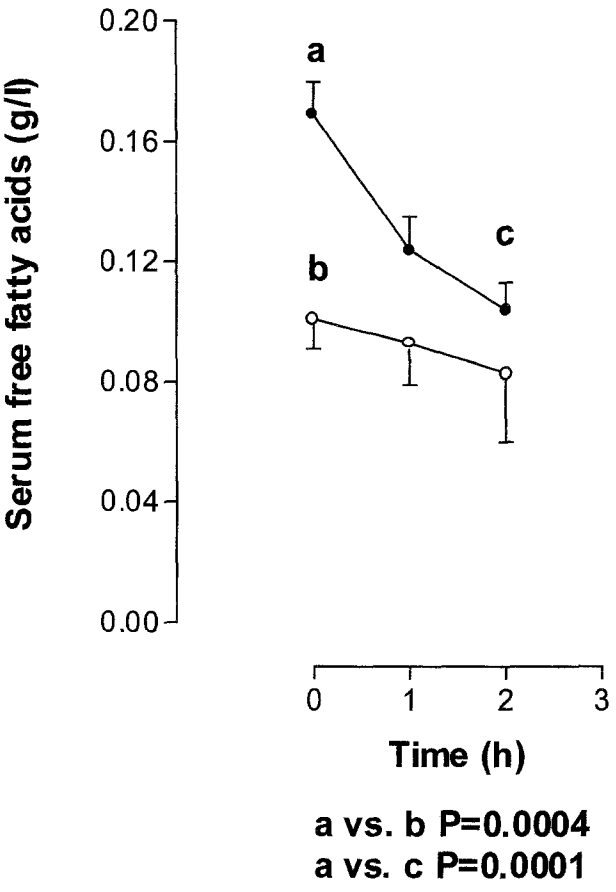

Fig 4. Serum free fatty acids before and after a standard oral glucose dose (75 g) in preeclamptic women (●) and normotensive pregnant control women (○). Data are the mean ± SE.

pathogenesis or clinical signs of preeclampsia. One explanation could be the effect of insulin resistance and concomitant changes in lipids and lipoproteins[7,8] on endothelial-cell function favoring vasoconstriction.[25] Insulin resistance may induce an increase in BP by the same mechanisms as in nonpregnant subjects, including an increase in catecholamines and in tubular sodium reabsorption.[26]

In conclusion, preeclamptic women are characterized by a state of increased insulin resistance. This may exist even before pregnancy, a fact supported by an increased incidence of preeclampsia in obese women[27] and in women with gestational diabetes[28] or polycystic ovarian disease characterized by insulin resistance.[29] Our present findings of increased insulin resistance 3 months after delivery and the presence of hyperinsulinemia[9] or hyperandrogenism[10] up to 17 years after preeclamptic pregnancy demonstrate that preeclamptic women are characterized by insulin resistance also in the nonpregnant state. The excess of cardiovascular disease in women with prior preeclamptic pregnancy[3,4] could thus also be explained.

ACKNOWLEDGMENT

We thank Leena Järvinen, Marja-Leena Pekonen, and Terhi Hakala for technical assistance, and Anna-Liisa Vuohijoki for organizing the sampling routines.

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