Midpregnancy Serum C-Peptide Concentration and Subsequent Pregnancy-Induced Hypertension

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OBJECTIVE — To test the hypothesis that elevated midpregnancy serum insulin (IRI) and C-peptide (CP) concentrations are associated with later development of pregnancy-induced hypertension (PIH), independent of prepregnancy obesity and midpregnancy blood pressure.

RESEARCH DESIGN AND METHODS — In this prospective study, a cohort of normotensive women, ages ≥18 years, performed a 50-g glucose challenge test at 24–30 weeks' gestational age. Blood samples were collected after an overnight fast and 1 h after glucose ingestion. Serum IRI and CP concentrations were measured in each sample. Maternal height, blood pressure, and proteinuria were measured at the time of glucose challenge testing and after 36 weeks' gestational age.

RESULTS — Of 320 subjects enrolled, 44 women (13.8%) had subsequent PIH. Crude odds ratios (ORs) for development of PIH associated with each 1 U rise in log fasting IRI, log fasting CP, and glucose-induced increase in CP (expressed as log [postprandial CP/fasting CP]) were 2.0 (95% CI 1.3–3.3), 1.8 (CI 1.2–2.7), and 2.3 (CI 1.1–4.9), respectively. After controlling for prepregnancy BMI, gestational age, and midpregnancy mean arterial pressure, adjusted ORs corresponding to log fasting IRI and CP for the development of PIH were 1.3 (95% CI 0.7–2.3) and 1.7 (CI 1.1–2.7), respectively, and, after adjustment for fasting CP, the adjusted OR of the glucose-induced rise in log CP was 3.7 (CI 1.5–9.3).

CONCLUSIONS — Mid-pregnancy fasting and postoral glucose CP levels are associated with subsequent development of PIH, independent of maternal obesity and midpregnancy baseline blood pressure. These findings may reflect an amplified β-cell response to glycemic stimulus, similar to that found in states of insulin resistance, that appears to be independently associated with PIH.

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I nsulin resistance and related hyperinsulinemia are associated with essential hypertension in nonpregnant individuals (1). Hyperinsulinemia is also linked with obesity, glucose intolerance, hypertension, atherosclerotic cardiovascular disease, and dyslipidemia, labeled the "insulin resistance syndrome" (1,2). Insulin resistance is characterized by decreased sensitivity of glucose uptake to insulin. The resulting compensatory hyperinsulinemia (3), reflected in elevated fasting and postprandial insulin and C-peptide (CP) concentrations, has been associated with insulin resistance (4–7).

Physiological insulin resistance is noted during late pregnancy, and patients with gestational diabetes show more insu-

lin resistance compared with pregnant control subjects with normal glucose tolerance (8). Pregnancy-induced hypertension (PIH), identified in ~10% of pregnancies (9), is associated with an increased incidence of perinatal morbidity and mortality (10). Its incidence is higher in gravidas with glucose intolerance (11). PIH may predict an increased risk for hypertension (12) and cardiovascular disease (13) in later life.

PIH and/or pre-eclampsia have been associated with hyperinsulinemia in both cross-sectional study designs (14) and cohort studies (15,16), although one study (17) did not support this association. More recent studies have identified insulin resistance in women with PIH (18) and pre-eclampsia (19) using the hyperinsulinemic-euglycemic clamp method.

On the other hand, maternal obesity and midpregnancy blood pressure are both associated with maternal hyperinsulinemia and development of PIH. No studies have addressed the possible confounding effect of maternal obesity and midpregnancy blood pressure on the association between maternal hyperinsulinism and PIH.

We hypothesized that *1*) fasting hyperinsulinemia (elevated immunoreactive insulin [IRI] or CP concentrations) at 24–30 weeks' gestational age is associated with a higher probability of subsequent development of PIH, and *2*) the association is independent of obesity and midpregnancy blood pressure.

RESEARCH DESIGN AND
METHODS — We enlisted gravidas ages ≥18 years and at 24–30 weeks' gestation from a universal screening program for gestational diabetes. Women who had prepregnancy hypertension, prepregnancy diabetes or prior gestational diabetes, chronic renal disease, thyroid disease, drug abuse, or multifetal pregnancy were excluded. Subjects whose systolic blood pressure was ≥140 mmHg or diastolic pressure was ≥90 mmHg before or at the time of midpregnancy glucose challenge were also excluded. Subjects gave written

Table 1—*Demographics and characteristics of study subjects*

	Value	Range
Age (years)	24.2 ± 5.3	18–42
Primipara	142 (43.0)	—
Prepregnancy BMI (kg/m^2)	25.0 ± 6.2	15.9–57.2
Gestational age at screening (weeks)	27.0 ± 1.3	24–30
MAP at glucose challenge (mmHg)	77 ± 7	55–101
MAP at term (mmHg)	82 ± 7	63–113

Data are means ± SD or n (%).

informed consent as per the Women & Infants' Hospital Institutional Review Board.

Between 24 and 30 weeks' gestation, subjects visited the clinic after an overnight fast of 10–12 h. After subjects rested for 5 min in the sitting position, the investigator (I.Y.) measured their blood pressure manually, using an appropriate size cuff and anaerobic sphygmomanometer according to the procedures recommended by the American Heart Association (20). Subjects' blood pressures were measured twice, 5 min apart, and the mean of the two measurements was used as the blood pressure observation.

Height, prepregnancy body weight, and current body weight were documented. Each subject's prepregnancy BMI was calculated from the report of her prepregnancy weight and our measurement of height (kg/m^2). We chose prepregnancy rather than midpregnancy BMI as a measure of adiposity because of the uncertain association of this variable with adiposity in the context of gestational changes. Analysis of the substitution of prepregnancy BMI for mid-pregnancy BMI showed no effect on the association of IRI and CP (fasting and postprandial increase) with PIH.

Blood samples were drawn from the antecubital vein. The first sample was collected in the fasting state and the second 1 h after subjects drank a 150-ml aqueous solution of 50 g of glucose. Samples for plasma glucose assay were collected in noncoated tubes containing 10 mg of potassium oxalate and 12.5 mg of sodium fluoride. These were iced immediately, centrifuged, and assayed within 2 h by the ultraviolet-hexokinase method (Synchron Systems, Beckman Instruments, Fullerton, CA). Samples for serum IRI and CP were collected in silicone-coated tubes, iced immediately, centrifuged, and

stored at −70°C until analysis. Both serum IRI and CP concentrations were determined by competitive radioimmunoassays (Diagnostic Systems Lab, Webster, TX).

Beginning at 36 weeks' gestation, blood pressure was measured weekly, after subjects rested in the sitting position for ≥5 min. PIH was defined as having systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg before the onset of labor, based on at least two assessments >6 h apart. Pre-eclampsia was defined as PIH in patients with proteinuria (defined as either 300 mg of urinary protein in 24 h or 2+ on Ames Dipstix from at least two random urine specimens collected >6 h apart).

Sample size was determined as follows. In the nonhyperinsulinemic group, defined as fasting IRI ≤75th percentile, we assumed that 5% of women would eventually develop PIH. We also assumed that variables such as prepregnancy BMI, mean arterial pressure (MAP), and estimated gestational age at entry would confound the relationship between hyperinsulinemia and development of PIH; roughly, the increase in sample size needed to overcome confounding effects was inversely proportional to $1 − r^2$, where r is the multiple correlation coefficient relating hyperinsulinemic status to the confounders. Under a 5% type I error rate (α), assuming no confounding effects, we calculated that 298 subjects were needed to detect, with 80% of power, a relative increase of three in the odds of PIH for subjects with hyperinsulinemia. We assumed $r = 0.25$, giving a target sample size of 318.

For analytic purposes, we used natural log transformations of endocrine variables. To examine the pairwise associations among variables, we calculated pairwise correlations first and then made adjustment for confounding variables us-

ing a multiple regression model. We used logistic regression analysis to test the association between each predictive variable and the development of PIH. When we tested the association of the postprandial rise in insulin and CP with PIH, we adjusted for fasting values of those variables. We also modeled probability of PIH as a function of the independent variables (prepregnancy BMI, gestational age, and MAP at entry) using logistic regression. For our hypothesis tests, two-sided significance levels at $P = 0.05$ were accepted.

RESULTS — Of 385 participants, 65 were excluded from analysis because of preterm delivery before 36 weeks' gestation ($n = 14$), pre-existing hypertension ($n = 13$), inappropriate blood sampling either at the fasting state or after 1-h glucose challenge ($n = 13$), incomplete medical records ($n = 9$), delivery in another place ($n = 7$), inappropriate gestational age at glucose challenge testing ($n = 5$), twin gestation ($n = 2$), and suspected endocrinopathy ($n = 2$). Analysis was based on the remaining 320 subjects. Maternal demographic data are summarized in Table 1. Subjects' racial backgrounds were as follows: 39% Hispanic, 38% white, 19% African American, and 4% other ethnic backgrounds, which was consistent with the overall racial distribution among patients at the Women and Infants' Hospital Antenatal Clinic. The distribution of values of each metabolic variable in the 320 subjects is summarized in Table 2. Only one subject was determined to have a significantly elevated fasting glucose concentration (128 mg/dl). She was treated with insulin and had no hypertension during pregnancy. The mean fasting glucose concentration among the other 10 subjects with gestational diabetes was 87 mg/dl (range 69–102), and only one of them later developed PIH.

Table 3 documents the correlation coefficients among four endocrine variables (log fasting IRI, log fasting CP, and the absolute post−glucose challenge rise in log IRI and log CP) and MAP at the time of glucose challenge testing. Log fasting IRI was significantly associated with the other three endocrine variables and the glucose challenge MAP. Log fasting CP was also significantly associated with MAP.

Table 4 summarizes regression coefficients describing the relationship between MAP at 24–30 weeks' gestation

Table 2—*Percentile values of insulin, C-peptide, and glucose*

	Percentile			
	25th	50th	75th	Range
Fasting immunoreactive insulin (pmol/l)	29.5	57.0	88.0	9.0–295
Postprandial increase in IRI (pmol/l)	156.0	232.0	340.0	−93–1,962
Fasting CP (nmol/l)	0.35	0.59	1.05	0.05–5.54
Postprandial increase in CP (nmol/l)	0.76	1.62	2.74	−0.98–12.25
Fasting glucose (mmol/l)	4.1	4.3	4.6	3.3–7.1
Postprandial glucose (mmol/l)	5.0	5.9	7.0	2.9–13.7

and each of the four endocrine variables. We fit one model for each measure of insulin secretion while controlling in each model for prepregnancy BMI and estimated gestational age at entry. Each regression coefficient reflects the mean difference in MAP at entry, corresponding to a 1-U difference in the log value of each endocrine variable. After controlling for prepregnancy BMI and gestational age, only log fasting IRI was significantly associated with midpregnancy MAP.

Of the 320 subjects, 44 (13.8%) developed PIH, including 9 who developed pre-eclampsia (2.8%). All endocrine variables were significantly associated with later development of PIH. For each unit rise in the log value of an endocrine variable, the crude odds ratios (ORs) were as follows: log fasting IRI, OR = 2.0 (95% CI 1.3–3.3); log postprandial IRI/fasting IRI, OR = 1.1 (CI 0.6–1.9.); log fasting CP, OR = 1.8 (CI 1.2–2.7); and log postprandial CP/fasting CP, OR = 2.3 (CI 1.1–4.9).

We then fit logistic regression models to estimate the effects of the insulin secretion measures after adjusting for prepregnancy BMI, gestational age, and midterm MAP. Because substitution of either systolic or diastolic blood pressure, or their combination, for MAP did not change the association between CP and PIH, and because midpregnancy MAP had a slightly higher association with PIH than the other blood pressure variables, we chose to use MAP in the regression model. Table 5 summarizes the fitted models, and reports adjusted ORs with 95% CI for each measure of insulin secretion. In this analysis, we found that both log fasting CP and change in log CP (from the fasting to the postprandial states) had a significant positive association with subsequent development of PIH. A log-unit difference in midpregnancy CP was associated with a 70% increase in the odds of PIH (OR = 1.7[1.1–2.7]), and a 1-U glucose-stimulated increase in log CP corresponded to a nearly fourfold increase in the odds of PIH (OR = 3.8 [CI 1.5–9.6]). IRI was positively associated with the development of PIH (for 1 log-unit increase, OR = 1.3 [0.7–2.7]), but the effect was not statistically significant.

Excluding subjects with gestational diabetes from the multiple logistic regression analysis did not change CP ORs for PIH (fasting CP OR of PIH = 1.7 [95% CI 1.1–2.7]; postprandial increase in CP = 3.6 [1.4–9.2]). The fasting and postprandial glucose concentrations among subjects developing subsequent PIH were 82 ± 7 and 118 ± 28 mg/dl, respectively; values among subjects who did not develop subsequent PIH were 79 ± 8 and 109 ± 27 mg/dl, respectively.

CONCLUSIONS — Fasting and postprandial hyperinsulinemia have been known to be associated with chronic hypertension in nonpregnant individuals (21). This observation may reflect an association between insulin resistance (determined by the hyperinsulinemic-euglycemic glucose clamp method) and essential hypertension (1,22).

Obesity may confound the association between hypertension and insulin resistance (1). Some investigations have found an association of fasting and postprandial insulin concentration with hypertension independent of obesity (21). Others, however, have found that the association between insulin concentration and blood pressure disappeared when the effects of age and obesity were taken into account (23).

Pregnancy provides an opportunity to examine the association between increased β-cell secretion and developing high blood pressure in subjects with no history of hypertension. Cross-sectional studies of third trimester fasting (14), postprandial hyperinsulinemia (24), and insulin resistance (18) have identified those conditions as being associated with new onset hypertension in late pregnancy.

Cohort studies during pregnancy have been inconclusive regarding the effect of maternal obesity on the association between hyperinsulinemia and subsequent PIH. Midgestation fasting hyperinsulinemia has been found to predict subsequent development of pre-eclampsia (proteinuria and hypertension) in African-American gravidas (15) and PIH in a Japanese cohort at risk for gestational diabetes (16). Both cohort studies found this association to be independent of prepregnancy BMI and gestational age. In contrast, others have found that hyperinsulinemia at 26–28 weeks' gestation was not predictive of PIH after controlling for BMI, race, and age (17). Consequently, we sought to examine possible confounding of maternal obesity and midpregnancy

Table 3—*Correlation coefficient matrix: midpregnancy endocrine variables and midpregnancy MAP*

	Log[FIRI (mU/ml)]	Log[PPIRI/FIRI]	Log[FCP (ng/ml)]	Log[PPCP/FCP]
Log[PPIRI/FIRI]	−0.586 (P < 0.0001)	—	—	—
Log[FCP (ng/ml)]	0.305 (P < 0.0001)	−0.088 (P = 0.11)	—	—
Log[PPCP/FCP]	−0.315 (P < 0.0001)	0.635 (P < 0.0001)	−0.294 (P < 0.0001)	—
MAP at glucose challenge (mmHg)	0.301 (P < 0.0001)	−0.086 (P = 0.12)	0.131 (P = 0.03)	−0.088 (P = 0.14)

FCP, fasting CP; FIRI, fasting IRI; PPCP, postprandial CP; PPIRI, postprandial IRI.

Table 4—*Regression coefficients characterizing the relationship between MAP at 24–30 weeks' gestation and four measurements relating to insulin secretion by normal-error linear regression models*

	Model			
	I	II	III	VI
Log[FIRI (μU/ml)] (95% CI)	2.30* (1.10–3.50)	2.69† (1.23–4.15)	—	—
Log[PPIRI/FIRI] (95% CI)	—	0.97 (−0.40–2.33)	—	—
Log[FCP (ng/ml)] (95% CI)	—	—	0.62 (−0.33–1.58)	0.56
Log[PPCP/FCP]) (95% CI)	—	—	—	−0.36 (−1.98–1.25)
Prepregnancy BMI (kg/m^2)	0.24†	0.24*	0.33‡	0.33‡
Gestational age at screening (weeks)	0.36	0.31	0.41	0.39

Data are means (95% CI). FCP, fasting CP; FIRI, fasting IRI; PPCP, postprandial CP; PPIRI, postprandial IRI. *$P < 0.001$; †$P < 0.005$; ‡$P < 0.0001$.

blood pressure on the association of insulin and CP with subsequent hypertension. Data from the present study suggest that amplified midpregnancy β-cell secretory activity (as reflected in fasting and postprandial CP concentrations) is associated with subsequent development of PIH, and that this association is independent of obesity and midpregnancy blood pressure.

Whether this CP and later PIH association can be ascribed to altered insulin metabolism or to insulin resistance is unclear. A negative correlation exists between insulin sensitivity and insulin secretion. Glucose disappearance after intravenous insulin infusion (the insulin tolerance test) is an indirect measure of insulin sensitivity and has been found to be associated with fasting peripheral insulin and CP concentrations in obese, euglycemic subjects (6) and among subjects

in a study of stroke risk factors in Japan (5). Kahn et al. (25) identified a hyperbolic negative correlation between insulin secretion and sensitivity in nondiabetic subjects using Bergman's minimal model technique. Insulin sensitivity, as determined by the hyperinsulinemic-euglycemic clamp technique, has also been shown to be negatively associated with fasting insulin and CP concentrations (7). These observations suggest that the association of fasting and postprandial CP concentrations with later PIH found in this study, independent of obesity, may manifest the underlying association between insulin resistance and the development of new-onset hypertension in late pregnancy.

We found that the apparent association of midpregnancy fasting insulin concentration with later development of PIH disappeared after confounding by midpregnancy blood pressure was accounted

for. The discrepant association of PIH with CP but not with fasting insulin may reflect the variability of hepatic clearance of insulin among subjects. Time-related changes in peripheral CP concentrations have been correlated with and used to calculate insulin release (26). However, insulin extraction by the liver may be affected by body fat and its distribution (27) and may contribute to confounding by maternal obesity of the insulin-PIH association. The insulin-PIH association may also be confounded by an underlying subclinical hypertension, resulting, in our study, in higher midpregnancy blood pressure among those with higher fasting insulin concentrations.

In summary, our data document that high midpregnancy fasting and postprandial CP concentrations are associated with subsequent development of new-onset PIH, and that this association is independent of obesity and midpregnancy blood pressure. These findings may reflect an amplified β-cell response to a glycemic stimulus, similar to that found in states of insulin resistance, that appears to be independently associated with PIH.

Table 5—*Logistic regression parameter estimates (OR) and 95% (CIs) for four probability models of pregnancy-induced hypertension*

	Model			
	I	II	III	VI
Log[FIRI (μU/ml)]	0.27	0.21	—	—
OR (95% CI)	1.3 (0.7–2.7)	—	—	—
Log[PPIRI/FIRI]	—	−0.13	—	—
OR (95% CI)		0.88 (0.5–1.6)	—	—
Log[FCP (ng/ml)]	—	—	0.54†	0.88‡
OR (95% CI)			1.7 (1.1–2.7)	—
Log[PPCP/FCP]	—	—	—	1.3§
OR (95% CI)				3.8 (1.5–9.6)
MAP at screening (mmHg)	0.13*	0.13*	0.14*	0.14*
Prepregnant BML (kg/m^2)	0.02	0.02	0.02	0.02
Gestational age at screening (weeks)	−0.14	−0.14	−0.16	−0.11

Data are means, with ORs (95% CI) shown under the estimated measurements of insulin secretion. FCP, fasting CP; FIRI, fasting IRI; PPCP, postprandial CP; PPIRI, postprandial IRI. *$P < 0.0001$; †$P < 0.02$; ‡$P < 0.002$; §$P = 0.005$.

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