Maternal Serum Prolactin and Prediction of Postpartum β-Cell Function and Risk of Prediabetes/Diabetes

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PATHOPHYSIOLOGY/COMPLICATIONS

OBJECTIVE

The insulin resistance of mid- to late pregnancy poses a physiologic stress test for the pancreatic β-cells, which must respond by markedly increasing their secretion of insulin. This response is achieved through an expansion of β-cell mass induced by the hormones prolactin and human placental lactogen (HPL). Conversely, the furan fatty acid metabolite 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF) has recently emerged as a negative regulator of β-cell function in pregnancy. Given their respective roles in the β-cell response to the stress test of gestation, we hypothesized that antepartum prolactin, HPL, and CMPF may relate to a woman's underlying glucoregulatory physiology and hence to her metabolic status after pregnancy.

RESEARCH DESIGN AND METHODS

Three hundred and sixty-seven women underwent measurement of fasting serum prolactin, HPL, and CMPF in the late-2nd/early-3rd trimester, followed by an oral glucose tolerance test (OGTT) at 3 months postpartum that enabled assessment of glucose tolerance, insulin sensitivity/resistance, and β-cell function (Insulin Secretion-Sensitivity Index-2 [ISSI-2]).

RESULTS

The postpartum OGTT identified 301 women with normal glucose tolerance (NGT) and 66 with prediabetes or diabetes. Serum prolactin in pregnancy was higher in women with postpartum NGT compared with those with postpartum prediabetes/diabetes (mean 98.2 vs. 80.2 ng/mL, $P = 0.0003$), whereas HPL and CMPF did not differ between the groups. On multiple linear regression analyses, antepartum prolactin was an independent determinant of postpartum ISSI-2 (β = 0.0016, $t = 2.96$, $P = 0.003$). Furthermore, higher serum prolactin in pregnancy independently predicted a lower risk of postpartum prediabetes/diabetes (odds ratio 0.50, 95% CI 0.35–0.72, $P = 0.0002$).

CONCLUSIONS

Serum prolactin in pregnancy predicts postpartum β-cell function and risk of prediabetes/diabetes.

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The latter half of pregnancy is a state of marked insulin resistance that poses a physiologic stress test for the pancreatic β-cells (1). For normal glucose homeostasis to be maintained in pregnancy, the β-cells must compensate for this insulin resistance by markedly increasing the secretion of insulin (1). An insufficient compensatory response will result in maternal hyperglycemia, as occurs in the setting of gestational diabetes mellitus (GDM) (1,2). This insufficient response is indicative of an underlying defect in β-cell function in women who develop GDM that is also the pathophysiologic basis for their high risk of postpartum progression to prediabetes and type 2 diabetes (T2D) in the years thereafter (3–6). Indeed, β-cell dysfunction is the central defect in the pathophysiology of both GDM and T2D (2,7). As such, the adaptive response of the β-cells to the physiologic stress test of pregnancy may provide unique insight into a woman's lifetime risk of diabetes (1).

Normal islet adaptation in pregnancy is believed to be dependent upon a marked expansion of β-cell mass that is stimulated by the circulating hormones prolactin and human placental lactogen (HPL) (8–11). Preclinical models have demonstrated that prolactin and placentally derived HPL both bind to the prolactin receptor on the β-cells and induce a series of downstream intracellular mediators that ultimately stimulate β-cell growth and proliferation (8–11). Indeed, the prolactin receptor has been shown to be essential for the expansion of β-cell mass in pregnancy (12,13). Whereas prolactin and HPL are key determinants of this normal physiologic response, the furan fatty acid metabolite 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF) was recently identified as a negative regulator of insulin secretion and hence a mediator of β-cell dysfunction in GDM (14,15). Given their respective roles in the β-cell response to the stress test of pregnancy, we hypothesized that these circulating factors may be relevant to a woman's underlying glucoregulatory physiology outside of gestation and hence to her risk of postpartum prediabetes and diabetes. Thus, our objective in this study was to evaluate the longitudinal associations of serum prolactin, HPL, and CMPF in pregnancy with β-cell function and glucose tolerance at 3 months postpartum in a cohort of women reflecting the full spectrum of gestational glucose tolerance (from normal to GDM) and hence a broad range of future diabetes risk.

RESEARCH DESIGN AND METHODS

This analysis was conducted in the setting of a prospective observational cohort study of early events in the natural history of T2D in which a cohort of women recruited at the time of antepartum screening for GDM is undergoing longitudinal metabolic characterization in pregnancy and the postpartum period. The study protocol has previously been described in detail (16). In brief, at our institution, all pregnant women are screened for GDM by 50-g glucose challenge test (GCT) late in the 2nd trimester, followed by referral for diagnostic oral glucose tolerance test (OGTT) if the GCT is abnormal (blood glucose ≥7.8 mmol/L at 1 h postchallenge). For this study, healthy women are recruited either before or after the GCT, and all participants then complete a 3-h 100-g OGTT (regardless of the GCT result). As previously described (6,16), the recruitment of women after an abnormal GCT serves to enrich the study population for those with GDM. At 3 months postpartum, participants return to the clinical investigation unit for reassessment of metabolic status by 2-h 75-g OGTT. The protocol has been approved by the Mount Sinai Hospital Research Ethics Board, and all women provide written informed consent for their participation. The current analysis was restricted to 367 women with singleton pregnancies in whom serum CMPF, HPL, and prolactin were measured at the antepartum OGTT and who had completed the 3-month postpartum visit.

Evaluation of Women in Pregnancy and at Three Months Postpartum

On the morning of the OGTT in pregnancy, interviewer-administered questionnaires were completed pertaining to medical, obstetrical, and family history. As previously described (16), the antepartum 3-h 100-g OGTT enabled ascertainment of gestational glucose tolerance status as follows: *1*) GDM, defined as two or more glucose values above the National Diabetes Data Group (NDDG) (17) diagnostic criteria on the OGTT (fasting blood glucose ≥5.8 mmol/L, 1-h glucose ≥10.6 mmol/L, 2-h blood glucose ≥9.2 mmol/L, or 3-h blood glucose ≥8.1 mmol/L); *2*) gestational impaired glucose tolerance (GIGT), defined as only one glucose value above NDDG thresholds; and *3*) normal glucose tolerance (NGT), defined as no glucose values above NDDG thresholds. Women diagnosed with GDM were referred to the diabetes-in-pregnancy clinic for clinical care, where they received glucose-lowering treatment in pregnancy, consisting of dietary/lifestyle counseling with or without antepartum insulin therapy.

At 3 months postpartum, participants returned for a 2-h 75-g OGTT, on which current glucose tolerance status was defined according to Canadian Diabetes Association guidelines (18). Prediabetes refers to impaired glucose tolerance (IGT), impaired fasting glucose (IFG), or combined IFG and IGT. At this visit, participants also underwent physical examination, with measurement of weight and waist circumference.

Laboratory Measurements on OGTT and Physiologic Indices

All OGTTs were performed in the morning after overnight fast, with venous blood samples drawn for the measurement of glucose and specific insulin at fasting and at 30, 60, and 120 min (and 180 min in pregnancy) after the ingestion of the glucose load. Specific insulin was measured with the Roche-Elecsys-1010 immunoassay analyzer and electrochemiluminescence immunoassay kit (Roche Diagnostics, Laval, Canada).

Glycemia was assessed with the area under the glucose curve ($AUC_{glucose}$) on the OGTT, calculated by trapezoidal rule. Insulin sensitivity was measured with the Matsuda index, an established measure of whole-body insulin sensitivity that has been validated against the euglycemic-hyperinsulinemic clamp (19). Insulin resistance (primarily hepatic) was evaluated with HOMA (HOMA-IR) (20). β-Cell function was assessed with the Insulin Secretion-Sensitivity Index-2 (ISSI-2), a validated measure of β-cell compensation that is analogous to the disposition index obtained from the intravenous glucose tolerance test, against which it has been directly validated (21,22). ISSI-2 is defined as the product of *1*) insulin secretion measured by the ratio of the $AUC_{insulin}$ to the $AUC_{glucose}$ and *2*) insulin sensitivity measured by

Matsuda index (21,22). A second measure of β-cell function was provided by the insulinogenic index divided by HOMA-IR (insulinogenic index/HOMA-IR), an established measure defined as the incremental change in insulin between 0 and 30 min divided by the incremental change in glucose over the same interval, divided by HOMA-IR (16).

Measurement of HPL, Prolactin, and CMPF

CMPF, HPL, and prolactin were measured from fasting serum at the OGTT in pregnancy. CMPF was measured by ELISA kit no. BG-HUM10440 from Novatein Biosciences (Woburn, MA). Samples were analyzed in 11 runs and were run without dilution. A serum pool was used as a calibrator, and the assigned value of 49 ng/mL was determined as the mean value from all runs. The within-run coefficient of variation (CV) averaged 16.5% for serum duplicates. HPL was measured by ELISA no. 20-HPLHU-E01 from Alpco (Salem, NH). Two quality-control pools were included in each run with mean 3.44 mg/L, CV 14.4%, and mean 15.0 mg/L, CV 15.4%. The manufacturer's standards were calibrated against the National Institute for Biological Standards and Control international standard for HPL International Reference Preparation (73/545). All pipetting of controls, samples, and standards was completed in 3 min with a Janus liquid handler equipped with an eight-tip Varispan arm (Perkin-Elmer, Woodbridge, Ontario, Canada). Prolactin was measured using the Meso Scale Discovery 96-well multiarray human prolactin assay (Meso Scale Diagnostics, Rockville, MD). A control serum pool had a mean of 3.49 ng/mL and CV 8.8% (n = 11 runs).

Statistical Analyses

All analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC). Continuous variables were tested for normality of distribution, and natural log transformations of skewed variables were used, where necessary, in subsequent analyses. The characteristics of women with NGT, prediabetes, and diabetes at 3 months postpartum were compared using the Kruskal-Wallis test for continuous variables and Fisher exact test for categorical variables (Table 1). For Tables 2 and 3, multiple linear regression analyses were performed to determine whether serum levels of CMPF, HPL, and prolactin in pregnancy could predict the following metabolic outcomes at 3

Table 1—Demographic, clinical, and metabolic characteristics of study population stratified into groups based on glucose tolerance status at 3 months postpartum

	NGT (n = 301)	Prediabetes (n = 60)	Diabetes (n = 6)	P
At OGTT in pregnancy				
Weeks' gestation (weeks)	30 (28–32)	29 (28–30)	28 (27–28)	0.010
Age (years)	34 (31–37)	35 (33–38)	36 (33–42)	0.075
Ethnicity (%)				0.047
White	72.1	58.3	66.7	
Asian	10.6	21.7	33.3	
Other	17.3	20.0	0	
Family history of DM (%)	48.8	66.7	33.3	0.023
Prepregnancy BMI (kg/m^2)	23.8 (21.5–27.5)	24.2 (21.4–27.6)	25.4 (22.5–28.1)	0.773
Gestational weight gain up to OGTT (kg)	10.3 (7.7–13.6)	10.5 (7.6–14.5)	5.0 (4.5–7.3)	0.045
Insulin sensitivity/resistance				
Matsuda index	4.7 (3.1–7.1)	3.4 (2.5–5.1)	7.3 (4.9–9.8)	0.001
HOMA-IR	1.6 (1.0–2.7)	2.1 (1.4–3.1)	1.2 (0.6–1.9)	0.013
β-Cell function				
ISSI-2	736 (563–912)	557 (481–707)	418 (348–583)	<0.0001
Insulinogenic index/HOMA-IR	10.3 (6.6–16.4)	6.8 (4.2–9.1)	3.3 (3.0–12.9)	<0.0001
Glucose tolerance on OGTT (%)				<0.0001
Normal	58.8	33.3	0	
GIGT	19.6	13.3	16.7	
GDM	21.6	53.3	83.3	
CMPF (ng/mL)	75.1 (57.7–101.5)	70.3 (55.5–99.6)	69.6 (53.2–98.0)	0.788
HPL (mg/L)	2.0 (1.5–3.7)	2.0 (1.5–3.1)	1.5 (1.2–2.6)	0.312
Prolactin (ng/mL)	93.4 (72.9–121.9)	82.7 (60.4–97.5)	79.2 (52.2–100.4)	0.004
At 3 months postpartum				
Time since delivery (months)	3 (3–4)	3 (3–3)	3.5 (3–4)	0.140
BMI (kg/m^2)	25.7 (22.9–29.1)	26.6 (24.4–30.9)	24.4 (22.5–27.4)	0.368
Waist circumference (cm)	88 (82–96)	90 (83–96)	79 (75–82)	0.106
Breast-feeding (%)	82.7	71.7	83.3	0.136
Insulin sensitivity/resistance				
Matsuda index	11.1 (7.2–15.8)	8.4 (5.4–10.7)	8.6 (7.0–11.6)	<0.0001
HOMA-IR	0.8 (0.6–1.3)	0.9 (0.6–1.5)	1.0 (0.7–1.4)	0.402
β-Cell function				
ISSI-2	805 (620–1025)	601 (505–666)	303 (265–355)	<0.0001
Insulinogenic index/HOMA-IR	10.7 (6.9–17.4)	7.5 (5.4–10.0)	3.8 (3.1–5.1)	<0.0001
OGTT results				
Fasting glucose (mmol/L)	4.5 (4.3–4.8)	4.6 (4.4–5.0)	4.6 (4.5–5.5)	0.135
$AUC_{glucose}$	12.7 (11.1–14.2)	16.4 (15.1–17.5)	20.8 (19.2–21.4)	<0.0001

Continuous variables are presented as median (interquartile range). Categorical variables are presented as proportions. DM, diabetes.

months postpartum: Matsuda index, HOMA-IR, ISSI-2, insulinogenic index/HOMA-IR, fasting glucose, and $AUC_{glucose}$. The models in Table 2 were adjusted for covariates from pregnancy as follows: model I shows associations adjusted for weeks' gestation at the antepartum OGTT, age, ethnicity, family history of diabetes, prepregnancy BMI, and gestational weight gain up to the OGTT; model II includes the covariates from model I in addition to adjustment for CMPF, HPL, and prolactin; and model III includes the covariates from model II in addition to adjustment for GDM. The models in Table 3 were adjusted for covariates from postpartum as follows: model I shows associations adjusted for age, ethnicity, family history of diabetes, postpartum BMI, breast-feeding, and time since delivery; model II includes additional adjustment for CMPF, HPL, and prolactin in pregnancy; model III includes further additional adjustment for GDM. Logistic regression analysis of (dependent variable) prediabetes/diabetes at 3 months postpartum was performed with the following covariates from pregnancy: weeks' gestation at the OGTT, age, ethnicity, family history of diabetes, prepregnancy BMI, gestational weight gain up to the OGTT, GDM, and serum CMPF, HPL, and prolactin in pregnancy (Fig. 1*A*). An analogous logistic regression analysis was performed with covariates from postpartum as follows: time since delivery, age, ethnicity, family history of diabetes, current BMI, breast-feeding, GDM in the recent pregnancy, and serum CMPF, HPL, and prolactin in pregnancy (Fig. 1*B*). The same approach was applied to logistic regression analyses of dependent variable prediabetes (Fig. 2).

RESULTS

Table 1 shows the demographic, clinical, and metabolic characteristics of the study population stratified into three groups based on glucose tolerance status at 3 months postpartum: *1*) women with NGT (n = 301), and *2*) women with prediabetes (n = 60, consisting of 57 with IGT, 2 with IFG, and 1 with IFG + IGT), and *3*) women with diabetes (n = 6). At the OGTT in pregnancy, among the women who went on to have postpartum prediabetes and diabetes, there was poorer β-cell function (ISSI-2 and insulinogenic index/HOMA-IR: both $P < 0.0001$) and a higher prevalence of GDM ($P < 0.0001$) than among those who had postpartum NGT. Of note, serum prolactin in pregnancy was significantly higher in the women with postpartum NGT compared with those with postpartum prediabetes and diabetes (median 93.4 vs. 82.7 vs. 79.2 ng/mL, respectively; P = 0.004), whereas the antepartum concentrations of CMPF and HPL did not differ between the three groups (P = 0.79 and P = 0.31, respectively). As shown in Supplementary Fig. 1, mean serum prolactin in pregnancy was higher in women with postpartum NGT compared with the women comprising each strata of postpartum prediabetes (IFG, IGT, and IFG + IGT) and those with diabetes. Accordingly, serum prolactin in pregnancy was significantly higher in the women with postpartum NGT compared with those with postpartum prediabetes/diabetes (mean 98.2 vs. 80.2 ng/mL, P = 0.0003).

At 3 months postpartum, the NGT, prediabetes, and diabetes groups did

Table 2—Adjusted associations of CMPF, HPL, and prolactin in pregnancy with outcomes (dependent variables) at 3 months postpartum

	CMPF			HPL			Prolactin		
	Coefficient	*t*	*P*	Coefficient	*t*	*P*	Coefficient	*t*	*P*
Log Matsuda index									
Model I	−0.0001	−0.47	0.638	0.1771	1.18	0.239	0.0005	0.70	0.486
Model II	−0.0001	−0.48	0.629	0.1771	1.13	0.259	0.0004	0.56	0.579
Model III	−0.0001	−0.48	0.635	0.0151	1	0.320	0.0004	0.55	0.582
Log HOMA-IR									
Model I	0.0004	1.19	0.235	−0.0285	−1.91	0.058	−0.0006	−0.77	0.443
Model II	0.0004	1.23	0.218	−0.0281	−1.86	0.064	−0.0004	−0.51	0.609
Model III	0.0004	1.23	0.218	−0.0284	−1.87	0.062	−0.0004	−0.51	0.609
Log ISSI-2									
Model I	0.0001	0.47	0.642	0.0067	0.60	0.551	0.0014	2.55	**0.011**
Model II	0.0002	0.71	0.475	0.0027	0.24	0.809	0.0014	2.57	**0.011**
Model III	0.0002	0.75	0.454	−0.0005	−0.04	0.965	0.0014	2.61	**0.010**
Log IGI/HOMA-IR									
Model I	−0.0001	−0.27	0.784	−0.0023	−0.09	0.930	0.0027	2.04	**0.042**
Model II	−0.00003	−0.07	0.947	−0.0089	−0.34	0.734	0.0027	2.05	**0.041**
Model III	−0.00003	−0.06	0.949	−0.0136	−0.52	0.601	0.0027	2.07	**0.039**
Fasting glucose									
Model I	0.0003	1.13	0.260	−0.0042	−0.33	0.744	−0.0005	−0.76	0.448
Model II	0.0003	1.07	0.288	−0.0049	−0.38	0.706	−0.0004	−0.63	0.526
Model III	0.0003	1.06	0.289	−0.003	−0.23	0.818	−0.0004	−0.63	0.530
$AUC_{glucose}$									
Model I	−0.0016	−1.05	0.294	0.0038	0.05	0.961	−0.0082	−2.17	**0.031**
Model II	−0.0019	−1.27	0.204	0.0246	0.32	0.749	−0.0087	−2.28	**0.023**
Model III	−0.002	−1.39	0.165	0.0633	0.88	0.381	−0.0085	−2.41	**0.017**

Model I: adjusted for age, ethnicity, family history of diabetes, prepregnancy BMI, gestational weight gain, and weeks' gestation at OGTT. Model II: adjusted for covariates in model I plus CMPF, HPL, and prolactin in pregnancy. Model III: adjusted for covariates in model II plus GDM. Boldface type indicates $P < 0.05$. IGI, insulinogenic index.

Table 3—Adjusted associations of CMPF, HPL, and prolactin in pregnancy with outcomes (dependent variables) at 3 months postpartum

	CMPF			HPL			Prolactin		
	Coefficient	*t*	*P*	Coefficient	*t*	*P*	Coefficient	*t*	*P*
Matsuda index									
Model I	0.00003	0.11	0.913	0.0141	1.01	0.313	0.0006	0.92	0.361
Model II	0.00003	0.11	0.910	0.0124	0.87	0.383	0.0006	0.83	0.406
Model III	0.00003	0.09	0.927	0.0092	0.65	0.515	0.0005	0.79	0.431
Log HOMA-IR									
Model I	0.0002	0.70	0.482	−0.0248	−1.77	0.078	−0.0006	−0.8	0.422
Model II	0.0002	0.76	0.448	−0.0237	−1.67	0.096	−0.0004	−0.58	0.559
Model III	0.0002	0.76	0.446	−0.0232	−1.63	0.104	−0.0004	−0.58	0.564
Log ISSI-2									
Model I	0.0002	0.75	0.454	0.0105	0.92	0.358	0.0016	2.96	**0.003**
Model II	0.0002	0.99	0.321	0.0053	0.46	0.644	0.0017	2.97	**0.003**
Model III	0.0002	0.98	0.326	0.0016	0.15	0.882	0.0016	2.96	**0.003**
Log IGI/HOMA-IR									
Model I	−0.0002	−0.04	0.972	0.0071	0.27	0.786	0.0031	2.41	**0.017**
Model II	0.0001	0.17	0.864	−0.0018	−0.07	0.946	0.0032	2.4	**0.017**
Model III	0.0001	0.15	0.884	−0.0079	−0.31	0.760	0.0031	2.39	**0.018**
Fasting glucose									
Model I	0.0002	0.66	0.508	−0.0028	−0.22	0.825	−0.0006	−0.96	0.336
Model II	0.0001	0.58	0.564	−0.0027	−0.22	0.829	−0.0005	−0.89	0.375
Model III	0.0001	0.60	0.547	−0.0005	−0.04	0.971	−0.0005	−0.85	0.395
$AUC_{glucose}$									
Model I	−0.002	−1.26	0.208	−0.0269	−0.34	0.731	−0.0106	−2.79	**0.006**
Model II	−0.0023	−1.51	0.132	0.0039	0.05	0.960	−0.0111	−2.89	**0.004**
Model III	−0.0022	−1.55	0.121	0.0488	0.67	0.503	−0.0106	−2.98	**0.003**

Model I: adjusted for age, ethnicity, family history of diabetes, BMI at 3 months postpartum, breast-feeding, and time since delivery. Model II: adjusted for covariates in model I plus CMPF, HPL, and prolactin in pregnancy. Model III: adjusted for covariates in model II plus GDM. Boldface type indicates $P < 0.05$. IGI, insulinogenic index.

not differ with respect to time since delivery, BMI, waist circumference, or breast-feeding. Compared with those with NGT, the women with prediabetes and diabetes had lower whole-body insulin sensitivity (Matsuda index: $P < 0.0001$). As anticipated, they continued to exhibit poorer β-cell function (ISSI-2 and insulinogenic index/HOMA-IR: both $P < 0.0001$) and greater glycemia ($AUC_{glucose}$: $P < 0.0001$) than the women with NGT.

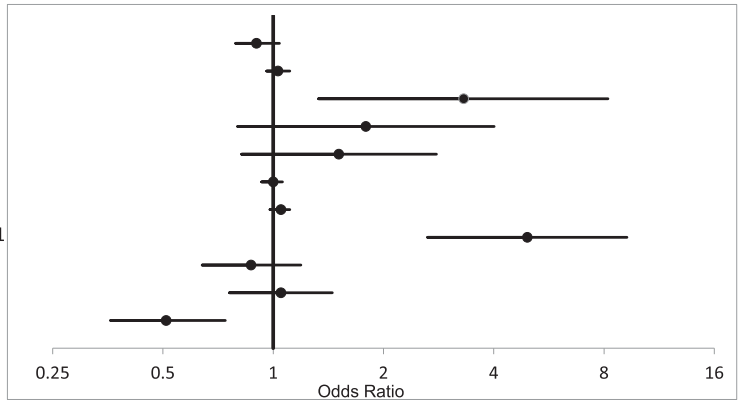
CMPF, HPL, and Prolactin in Pregnancy as Predictors of Postpartum Metabolic Function

We next performed a series of multiple linear regression analyses to determine whether serum levels of CMPF, HPL, and prolactin in pregnancy could predict postpartum metabolic function at 3 months after delivery. Table 2 shows multiple linear regression models evaluating the independent associations of each of these analytes with features of postpartum metabolic function (insulin sensitivity/resistance, β-cell function, glycemia) after adjustment for the following covariates in turn: model I consists of variables in pregnancy, including weeks' gestation at the antepartum OGTT, age, ethnicity, family history of diabetes, prepregnancy BMI, and gestational weight gain up to the OGTT; model II includes additional adjustment for CMPF, HPL, and prolactin; and model III includes further additional adjustment for GDM. These analyses revealed that CMPF, HPL, and prolactin were not significant determinants of postpartum whole-body insulin sensitivity (Matsuda index) or insulin resistance (HOMA-IR). Importantly, however, prolactin in pregnancy consistently emerged as a significant independent predictor of postpartum β-cell function in all models of ISSI-2 (all $P \leq 0.011$) and insulinogenic index/HOMA-IR (all $P \leq 0.042$). Furthermore, higher antepartum prolactin predicted lower $AUC_{glucose}$ on the postpartum OGTT in all models (all $P \leq 0.031$). CMPF and HPL were not associated with any of these outcomes. It thus emerges that antepartum serum prolactin is an independent determinant of better β-cell function and lower glycemia at 3 months postpartum after complete adjustment for covariates in pregnancy, including GDM.

We then constructed models to address whether prolactin in pregnancy predicts these outcomes after adjustment for postpartum covariates. Table 3 shows multiple linear regression models evaluating the antepartum analytes CMPF, HPL, and prolactin using an approach to model construction analogous to that in Table 2 but with adjustment for postpartum factors as follows: model I shows associations adjusted for age, ethnicity, family history of diabetes, postpartum BMI, breast-feeding, and time since delivery; model II includes additional adjustment for CMPF, HPL, and prolactin in pregnancy; and model III includes further additional adjustment for GDM. The findings from these analyses (Table 3) were unchanged from those in Table 2, with prolactin in pregnancy consistently emerging in all models as a significant independent predictor of β-cell function (ISSI-2, all $P = 0.003$, and insulinogenic index/HOMA-IR, all $P \leq 0.018$) and lower $AUC_{glucose}$ (all $P \leq 0.006$).

A **Model with predictors from pregnancy only**

	OR	95% CI	P
Weeks' gestation at OGTT	0.90	[0.79, 1.04]	0.149
Age	1.03	[0.96, 1.11]	0.389
Asian ethnicity	3.31	[1.33, 8.20]	0.010
Other non-white ethnicity	1.79	[0.80, 4.01]	0.159
Family history of diabetes	1.51	[0.82, 2.79]	0.191
Prepregnancy BMI	1.00	[0.93, 1.06]	0.881
Gestational weight gain	1.05	[0.98, 1.11]	0.166
GDM	4.94	[2.64, 9.24]	<0.0001
CMPF in pregnancy (per SD)	0.87	[0.64, 1.19]	0.380
HPL in pregnancy (per SD)	1.05	[0.76, 1.45]	0.765
Prolactin in pregnancy (per SD)	0.51	[0.36, 0.74]	0.0003



B **Model with additional predictors from postpartum**

	OR	95% CI	P
Time since delivery	0.82	[0.56, 1.21]	0.315
Age	1.03	[0.96, 1.10]	0.479
Asian ethnicity	3.86	[1.54, 9.67]	0.004
Other non-white ethnicity	1.73	[0.77, 3.90]	0.183
Family history of diabetes	1.54	[0.83, 2.85]	0.169
Current BMI	1.01	[0.95, 1.07]	0.782
Breast-feeding	0.49	[0.24, 1.02]	0.057
GDM	4.32	[2.36, 7.90]	<0.0001
CMPF in pregnancy (per SD)	0.82	[0.59, 1.13]	0.217
HPL in pregnancy (per SD)	1.04	[0.76, 1.43]	0.796
Prolactin in pregnancy (per SD)	0.50	[0.35, 0.72]	0.0002

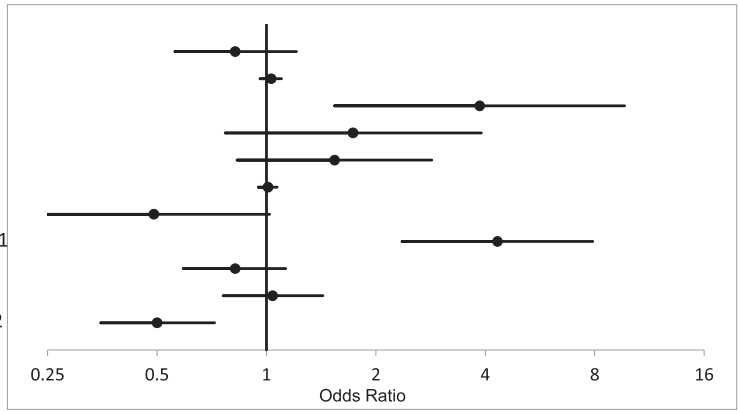


Figure 1—Logistic regression analyses of (dependent variable) prediabetes/diabetes at 3 months postpartum, using predictors from pregnancy only (*A*) and additional predictors from postpartum (*B*). The reference group for ethnicity variables is white ethnicity.

CMPF, HPL, and Prolactin in Pregnancy as Predictors of Postpartum Prediabetes/Diabetes

Logistic regression analyses were then performed to determine whether prolactin in pregnancy is a predictor of prediabetes/diabetes at 3 months postpartum (Fig. 1). In a model consisting only of predictors from pregnancy (Fig. 1*A*), higher antepartum serum prolactin was a significant independent determinant of a lower risk of postpartum prediabetes/diabetes (odds ratio [OR] 0.51, 95% CI 0.36 to 0.74, $P = 0.0003$). The only other significant predictors were GDM (OR 4.94, 95% CI 2.64 to 9.24, $P < 0.0001$) and Asian ethnicity (OR 3.31, 95% CI 1.33 to 8.20, $P = 0.01$). The *C* statistic for this model was higher than that of the model without CMPF, HPL, and prolactin in pregnancy (0.7998 vs. 0.7606, $P = 0.024$), indicating that these antepartum circulating factors significantly improved the prediction of postpartum prediabetes/diabetes. In addition, the significant predictors were unchanged with further adjustment for GIGT (data not shown).

These findings were unchanged in a model adjusting for postpartum factors (Fig. 1*B*), with serum prolactin in pregnancy again emerging as a significant independent predictor of a lower risk of prediabetes/diabetes at 3 months postpartum (OR 0.50, 95% CI 0.35 to 0.72, $P = 0.0002$). Again, the *C* statistic for this model was significantly higher than that of the model without the antepartum serum factors (0.7946 vs. 0.7420, $P = 0.0066$). In addition, the significant predictors were again unchanged with further adjustment for GIGT (data not shown).

Given the limited number of women with postpartum diabetes, the logistic regression models were repeated with dependent variable postpartum prediabetes (Fig. 2). In a model consisting only of predictors from pregnancy (Fig. 2*A*), higher antepartum serum prolactin remained a significant independent determinant of a lower risk of postpartum prediabetes (OR 0.50, 95% CI 0.35 to 0.73, $P = 0.0003$). The only other significant predictors were GDM (OR 4.56, 95% CI 2.40 to 8.69, $P < 0.0001$) and Asian ethnicity (OR 3.09, 95% CI 1.20 to 7.97, $P = 0.019$). Again, these findings were similar in a model with adjustment for postpartum factors (Fig. 2*B*), with serum prolactin in pregnancy remaining a significant independent predictor of a lower risk of prediabetes at 3 months postpartum (OR 0.51, 95% CI 0.35 to 0.73, $P = 0.0003$).

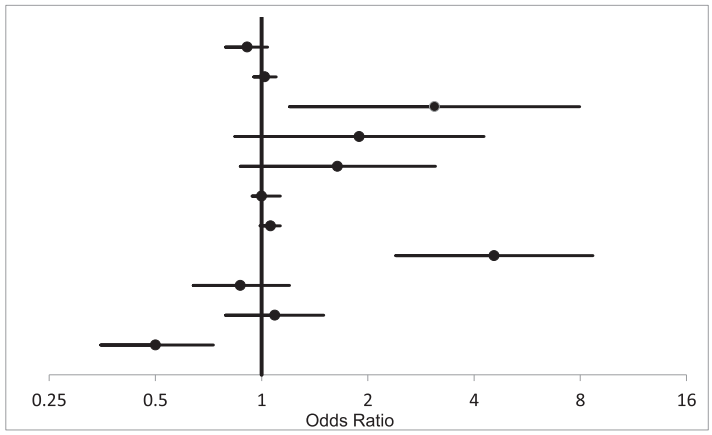
Serum prolactin in pregnancy <115 ng/mL had 84.9% sensitivity for predicting postpartum prediabetes/diabetes but with 30.8% specificity (Supplementary Table 1). As would be anticipated and consistent with the central role of β-cell dysfunction in the pathophysiology of diabetes, ISSI-2 in pregnancy <780 had better operating characteristics as a predictor of postpartum prediabetes/diabetes and slightly higher area under the receiver operating characteristic curve (0.70 vs. 0.63) (Supplementary Table 1).

CONCLUSIONS

In this study, we demonstrate that serum prolactin in pregnancy is higher in women who subsequently maintain NGT at 3

A **Model with predictors from pregnancy only**

	OR	95% CI	P
Weeks' gestation at OGTT	0.91	[0.79, 1.04]	0.172
Age	1.02	[0.95, 1.10]	0.586
Asian ethnicity	3.09	[1.20, 7.97]	0.019
Other non-white ethnicity	1.89	[0.84, 4.27]	0.124
Family history of diabetes	1.64	[0.87, 3.11]	0.129
Prepregnancy BMI	1.00	[0.94, 1.07]	0.993
Gestational weight gain	1.06	[0.99, 1.13]	0.074
GDM	4.56	[2.40, 8.69]	<0.0001
CMPF in pregnancy	0.87	[0.64, 1.20]	0.411
HPL in pregnancy	1.09	[0.79, 1.50]	0.610
Prolactin in pregnancy	0.50	[0.35, 0.73]	0.0003



B **Model with additional predictors from postpartum**

	OR	95% CI	P
Time since delivery	0.78	[0.52, 1.17]	0.219
Age	1.02	[0.95, 1.10]	0.588
Asian ethnicity	3.46	[1.33, 8.99]	0.011
Other non-white ethnicity	1.82	[0.81, 4.10]	0.150
Family history of diabetes	1.78	[0.93, 3.41]	0.080
Current BMI	1.01	[0.95, 1.08]	0.749
Breast-feeding	0.46	[0.22, 0.98]	0.043
GDM	3.75	[2.01, 7.01]	<0.0001
CMPF in pregnancy	0.82	[0.59, 1.14]	0.236
HPL in pregnancy	1.10	[0.80, 1.51]	0.566
Prolactin in pregnancy	0.51	[0.35, 0.73]	0.0003

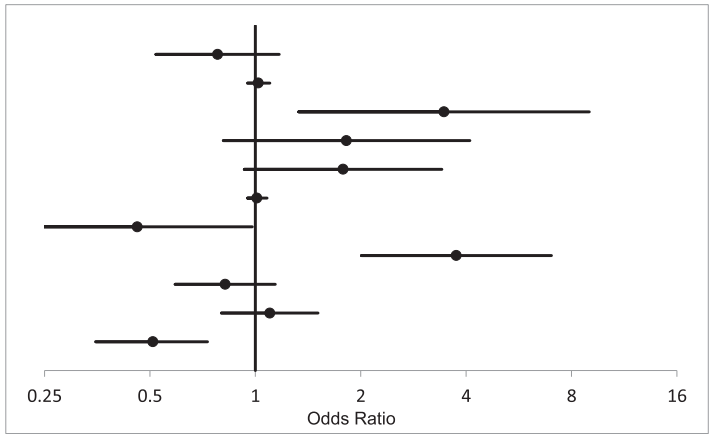


Figure 2—Logistic regression analyses of (dependent variable) prediabetes at 3 months postpartum, using predictors from pregnancy only (*A*) and additional predictors from postpartum (*B*). The reference group for ethnicity variables is white ethnicity.

months postpartum compared with those who have postpartum prediabetes/diabetes. On fully adjusted analyses, antepartum prolactin emerges as an independent determinant of postpartum β-cell function. Most importantly, higher serum prolactin in pregnancy independently predicts a lower risk of prediabetes/diabetes at 3 months after delivery. Thus, serum prolactin in pregnancy can provide insight into postpartum diabetes risk, likely through its implications for β-cell function.

Although best known for its lactogenic activity, prolactin is also recognized as a principal determinant of islet adaptation to pregnancy (8–11). Indeed, a substantial body of evidence from preclinical studies has shown that both prolactin and HPL bind to the prolactin receptor on β-cells and induce the expansion of β-cell mass through a series of downstream intracellular mediators, including tryptophan hydroxylase 1 (the rate-limiting enzyme in serotonin synthesis), cell-cycle regulators, survivin, forkhead proteins, and menin (9,23–26). Of note, the peak of β-cell proliferation coincides with increased circulating levels of prolactin and HPL, supporting the importance of these hormones in this context (9). Furthermore, the prolactin receptor to which they bind has been shown to be essential for the expansion of β-cell mass in pregnancy (12,13). Beneficial effects of prolactin on β-cell physiology may also extend to the nonpregnant state. Specifically, in cell culture systems, prolactin has been shown to increase β-cell proliferation, inhibit key caspases of the extrinsic and intrinsic pathways that lead to islet apoptosis, and promote β-cell survival (27–29). Moreover, prolactin supplementation of pretransplant culture media can improve β-cell survival in human islet preparations (30). Taken together, these data suggest that endogenous prolactin potentially could hold implications for β-cell function and overall glucose homeostasis outside of pregnancy.

To date, however, there has been only limited study of this question in humans. In a cross-sectional study of 2,377 individuals in China, those with higher circulating prolactin concentrations had better β-cell function (measured by HOMA) and a lower prevalence of prediabetes and diabetes (31). Similarly, in a study of 3,993 German adults, there was an inverse association between prolactin concentration and the prevalence of T2D in both men and women (32). In the setting of this background, the current study extends the literature in three key ways. First, we have evaluated prolactin during late pregnancy, representing a strategic point in time when it is playing a central role in the β-cell response to the stress test of gestation. Second, we demonstrate that, when measured in the setting of this physiologic challenge, serum prolactin is independently associated with subsequent β-cell function at 3 months postpartum (as assessed by both ISSI-2 and insulinogenic index/HOMA-IR) after

adjustment for diabetes risk factors both during and after pregnancy. Third and most importantly, the current study demonstrates a longitudinal independent relationship between higher serum prolactin in pregnancy and subsequent lower risk of postpartum prediabetes/diabetes.

While these data suggest that beneficial effects on β-cell function are likely responsible for the lower diabetes risk, the underlying mechanism remains unclear. One possibility is that the antepartum prolactin measurement is providing a surrogate indicator of the degree of β-cell mass expansion in pregnancy, which in turn may hold ongoing implications for insulin secretory capacity at 3 months postpartum. Although β-cell mass returns to pregravid levels shortly after delivery in rodent models (33), the precise time course of the analogous regression in humans remains to be established. Another possibility is that the prolactin response in driving islet adaptation to the stress test of pregnancy may relate to the beneficial β-cell effects that might be anticipated from lactation and its associated prolactinemia after delivery. In this regard, however, it should be noted that the independent associations of antepartum prolactin with postpartum β-cell function and glucose tolerance were all adjusted for breast-feeding status. Finally, a third possibility is that measurement of prolactin in the setting of the stress test of pregnancy (and its central role therein) may be providing unique insight into unrecognized elements of the underlying glucoregulatory physiology of the mother that contribute to her postpartum β-cell function and glucose tolerance. Our findings suggest that further study is needed to elucidate the glucose homeostatic implications of the serum prolactin concentration in pregnant women.

From a clinical perspective, it is noteworthy that the inverse relationship between serum prolactin in pregnancy and postpartum prediabetes/diabetes persisted after adjustment for classical diabetes risk factors (age, ethnicity, family history, weight, and breast-feeding status) and even GDM, which is a particularly powerful predictor of future risk of T2D (34). Indeed, coupled with the increased *C* statistic from the enhanced models, these data suggest that prolactin offers insight into postpartum diabetes risk above and beyond GDM status. This finding raises the possibility that prolactin measurement in pregnancy potentially could contribute to postpartum risk stratification in women with GDM by identifying those in whom it may be particularly important to ensure completion of the OGTT that is recommended in the first 6 months after delivery (35), owing to their enhanced risk of prediabetes/diabetes. The potential value of this additional risk stratification is underscored by the low rates of completion of this OGTT in current clinical practice, representing a missed opportunity for early diagnosis and intervention in this high-risk patient population (36). The mechanism by which prolactin provides insight into postpartum diabetes risk beyond GDM status is also of interest. In this regard, one possibility is that the antiapoptotic effects of prolactin (27–29) may play a role in modifying future diabetes risk in the setting of GDM, wherein hyperglycemia may otherwise contribute to β-cell death (37–39) and hence the risk of postpartum glucose intolerance. Further mechanistic study is needed in this regard.

A limitation of this study is that prolactin was not also measured at 3 months postpartum, such that the impact of circulating levels of prolactin isoforms at the time of the assessment of glucose tolerance cannot be determined. Similarly, measurement of prolactin prior to pregnancy would also have been of interest to determine whether the higher antepartum prolactin that was associated with lower risk of postpartum prediabetes/diabetes existed before pregnancy or if it was a reflection of greater antepartum elevation. However, pregravid assessment was not possible, since participants were recruited during pregnancy. Another limitation is that the modest number of women with diabetes at 3 months postpartum (n = 6) necessitated that, despite being distinct metabolic states, prediabetes and diabetes be combined into a single group in some of the analyses. Finally, although this study shows a robust relationship between higher serum prolactin in pregnancy and lower risk of prediabetes/diabetes at 3 months after delivery, it does not provide insight into diabetes risk beyond early postpartum. Further study is thus needed to determine whether antepartum prolactin relates to the long-term future risk of T2D in women.

In summary, serum prolactin in pregnancy is higher in women who subsequently maintain NGT at 3 months postpartum compared with those who have postpartum prediabetes/diabetes. Indeed, higher prolactin in pregnancy is an independent predictor of both better β-cell function and a lower likelihood of prediabetes/diabetes after complete adjustment for T2D risk factors. It thus emerges that serum prolactin in pregnancy is a previously unrecognized factor that can provide novel insight into postpartum diabetes risk in young women.

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Duality of Interest. R.R. is part holder of U.S. Provisional Patent Application No. 61/703,867 "CMPF as a biomarker for diabetes and associated methods." No other potential conflicts of interest relevant to this article were reported.
Author Contributions. R.R., P.W.C., A.J.H., M.S., and B.Z. designed and implemented the study. R.R., C.Y., and C.K.K. contributed to the analysis plan and interpretation of data. C.Y. performed the statistical analyses. R.R. wrote the first draft. All authors critically revised the manuscript for important intellectual content and approved the final manuscript. R.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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