Duration of Lactation and Maternal Adipokines at 3 Years Postpartum

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OBJECTIVE—Lactation has been associated with reduced maternal risk of type 2 diabetes, the metabolic syndrome, and cardiovascular disease. We examined the relationship between breastfeeding duration and maternal adipokines at 3 years postpartum.

RESEARCH DESIGN AND METHODS—We used linear regression to relate the duration of lactation to maternal leptin, adiponectin, ghrelin, and peptide YY (PYY) at 3 years postpartum among 570 participants with 3-year postpartum blood samples (178 fasting), prospectively collected lactation history, and no intervening pregnancy in Project Viva, a cohort study of mothers and children.

RESULTS—A total of 88% of mothers had initiated breastfeeding, 26% had breastfed ≥12 months, and 42% had exclusively breastfed for ≥3 months. In multivariate analyses, we found that duration of total breastfeeding was directly related to PYY and ghrelin, and exclusive breastfeeding duration was directly related to ghrelin (predicted mean for never exclusively breastfeeding: 790.6 pg/mL vs. ≥6 months of exclusive breastfeeding: 1,008.1 pg/mL; $P < 0.01$) at 3 years postpartum, adjusting for pregravid BMI, gestational weight gain, family history of diabetes, parity, smoking status, and age. We found a nonlinear pattern of association between exclusive breastfeeding duration and adiponectin in multivariate-adjusted models.

CONCLUSIONS—In this prospective cohort study, we found a direct relationship between the duration of lactation and both ghrelin and PYY at 3 years postpartum. ***Diabetes*** **60:1277–1285, 2011**

Type 2 diabetes causes substantial morbidity and mortality, affecting >9 million women in the U.S. Recent epidemiologic data (1–4) suggest that lactation may reduce a woman's risk for this disease. Lactation also has been associated with more favorable lipid profiles after weaning (5), reduced metabolic syndrome risk (6,7), and lower rates of hypertension (3,8) and myocardial infarction (3,9).

These findings suggest that lactation may be a modifiable risk factor for metabolic disease in women. We have previously hypothesized that lactation mobilizes maternal adipose stores, resetting maternal metabolism after pregnancy (10). Adipose tissue produces cytokines called adipokines, and these endocrine markers are associated with subsequent metabolic disease risk. High leptin levels are associated with adverse metabolic profiles (11,12), although these associations are attenuated with adjustment for fat mass. High ghrelin (13–16) and adiponectin (17–20) levels are associated with reduced diabetes and metabolic disease risk. The protein peptide YY (PYY) also plays a key role in metabolism and appetite regulation, and low PYY levels are associated with obesity (21,22). No studies, to our knowledge, have measured the association between lactation and maternal levels of leptin, adiponectin, ghrelin, or PYY after weaning. We therefore examined the association between lactation duration and these markers at 3 years postpartum in Project Viva, a prospective cohort study of maternal and infant health. We hypothesized that longer durations of lactation would be associated with lower leptin and higher adiponectin, ghrelin, and PYY.

RESEARCH DESIGN AND METHODS

Women were recruited for Project Viva at their first prenatal visit at one of eight urban and suburban obstetrical offices of a multispecialty group practice in eastern Massachusetts. To be eligible for the study, potential participants were required to be fluent in English, <22 weeks' gestation, and have a singleton pregnancy; 65% of eligible women were recruited. All participants provided written informed consent. The human studies committee of Harvard Pilgrim Health Care approved all procedures in accordance with ethical standards for human experimentation.

Of 2,128 participating women who gave birth, 1,579 were invited to a 3-year follow-up examination because they had completed dietary questionnaires during pregnancy; 761 of these women were eligible for the current analysis because they had not delivered another child since the birth of the index child 3 years previously, they did not have type 1 or type 2 diabetes, and they attended the 3-year visit. Of these women, 611 provided a blood sample. We excluded women missing breastfeeding duration (n = 30), gestational weight gain (n = 4), gestational diabetes (n = 4), or adipokine measurements (n = 3), leaving 570 women for analysis. Fasting blood samples were available for 175 of 570 women.

When we compared women who provided fasting samples with those who provided nonfasting samples, we found no differences in adiponectin, leptin, breastfeeding duration, prepregnancy BMI, gestational weight gain, age, race, parity, gestational glucose tolerance, or family history of diabetes. We excluded from our exclusive lactation analysis women missing data on the timing of introduction of formula or complementary foods (n = 130).

Assessment of lactation. At approximately 28 weeks' gestation, study participants provided information on their intention to breastfeed. Shortly after delivery, participants again reported their intention and initiation of breastfeeding. At the 6-month follow-up visit, we asked women whether they were breastfeeding and whether they had introduced formula or solid foods. Women reported timing of weaning and introduction of supplemental foods in months, weeks, or days. We similarly assessed breastfeeding at 12 months postpartum.

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For our categorical analysis, we coded duration of lactation as 0, >0 to <3, ≥3 to <6, ≥ 6 to <12, and ≥ 12 months. We defined exclusive lactation as time to first introduction of solid foods, formula, or juice. We coded duration of exclusive lactation in the categories of 0, >0 to <1, 1 to <3, 3 to <6, and ≥6 months.

We used data on duration of breastfeeding, reasons for weaning or supplementing, and mothers' planned duration reported at 28 weeks' gestation to define "curtailed breastfeeding" as *1*) weaning before 3 months among mothers who planned to breastfeed at 3 months or *2*) introduction of formula before 3 months among mothers who planned to exclusively breastfeed at 3 months. We reasoned that the curtailed breastfeeding group may have had physiologic lactation failures that did not affect women who had planned to wean before 3 months. At the 6-month interview, mothers who were not exclusively breastfeeding identified reasons for introducing formula from a structured list. We classified women as having problems with milk supply if they endorsed any of the following: "I wasn't producing enough breast milk to satisfy my baby," "I had difficulty or didn't like pumping breast milk," or "My baby was not gaining enough weight with breastfeeding." We used categories of never breastfeeding, curtailed breastfeeding with low milk supply, curtailed breastfeeding without low milk supply, and successful breastfeeding to assess whether low milk production was associated with maternal adipokine levels at 3 years postpartum.

Assessment of adipokines at 3 years postpartum. Women returned with their children at 3 years postpartum for a physical examination that included anthropometric measurements and a blood sample. We tested all blood samples for leptin and adiponectin. We identified as fasting those participants who did not eat or drink anything other than water for 8 h before blood samples were obtained. We tested fasting blood samples for ghrelin and PYY. Blood samples were collected by trained phlebotomists and transferred within 24 h for storage in liquid-nitrogen freezers. Leptin (intra-assay coefficient of variation [CV] 3.0–3.3%, interassay CV 3.5–5.4%, and sensitivity 7.8 pg/mL) and adiponectin (intra-assay CV 2.5–4.7%, interassay CV 5.8–6.9%, and sensitivity 0.89 ng/mL) were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). Total ghrelin (acylated and deacylated) was measured by a radioimmunoassay (Linco Research, St. Louis, MO) (intra-assay CV 3.3–10%, interassay CV 14.7–17.8%, and sensitivity 93 pg/mL). PYY was measured using an enzyme-linked immunosorbent assay (Diagnostic Systems Laboratory, Webster, TX) with a sensitivity of 2.1 pg/mL, an intra-assay CV of 3.0%, an interassay CV of 7.4%, and 100% cross-reactivity for the full-length peptide ($PYY_{1–36}$) and the truncated $PYY_{3–36}$, both of which have biological activity.

Study covariates. At the initial prenatal visit, participants reported sociodemographic variables, including parity, prepregnancy weight and height, and family history of type 2 diabetes. We calculated gestational weight gain as the difference between the last clinical weight recorded prior to delivery and the self-reported prepregnancy weight. Self-reported prepregnancy weight in study participants is highly correlated (r = 0.99) with clinically recorded prepregnant weight (23). Maternal gestational glucose tolerance was assessed clinically with the nonfasting 50-g oral glucose loading test (GLT) administered at 26–28 weeks' gestation. Women with a result of ≥140 mg/dL underwent a 100-g oral glucose tolerance test (OGTT), administered after an overnight fast. Normal results were defined by Carpenter-Coustan criteria (fasting <95 mg/dL, 1 h <180 mg/dL, 2 h <155 mg/dL, and 3 h <140 mg/dL). Gestational glucose tolerance was categorized as normal (GLT <140), transient hyperglycemia (GLT ≥140, GTT with no abnormal results), impaired glucose tolerance (GTT with one abnormal result), or gestational diabetes (GLT ≥140, GTT with two or more abnormal results). Women returned with their children at 3 years postpartum for a physical examination that included measured weight to the nearest 0.1 kg using a research-quality scale. Weight change related to pregnancy was calculated as the difference between the 3-year physical exam weight and self-reported prepregnancy weight. Hormonal contraceptive use since the index birth was assessed at the 6-year visit (missing for n = 210).

Data analysis. We used Spearman correlation to measure associations among adipokines. Prior to regression analysis, we log transformed PYY measurements because this variable was not normally distributed. To facilitate interpretation of both the magnitude and clinical significance of differences among lactation duration groups, we present results from our linear regression models as predicted values and 95% CIs for participants of average BMI who were white, aged 35–40 years, had two children, had no parental history of type 2 diabetes, had normal glucose tolerance, were nonsmokers, and gained 15 kg during the index pregnancy. For PYY, we present predicted geometric means and 95% CIs after exponentiation.

We used linear regression to model the relation between both total and exclusive lactation duration category and adipokines at 3 years. We used the partial F test to assess the significance of differences among lactation duration categories in predicting metabolic outcome. We included in our multivariate models those covariates that were associated with duration of breastfeeding or that were a priori risk factors for an adverse metabolic profile at 3 years postpartum. Smoking has been associated with differences in leptin and ghrelin levels (24,25), and we therefore included current smoking status in our multivariate-adjusted models. Because prepregnancy BMI had a nonlinear association with breastfeeding duration, we used a three-knot quadratic spline model (26) to adjust for prepregnancy BMI. Quadratic splines allow for more complete adjustment for potential confounders than a linear or categorical approach. In our multivariate models, we also adjusted for maternal age, race, parity, gestational weight gain, gestational glucose tolerance (normal, transient hyperglycemia, impaired glucose tolerance, or gestational diabetes), family history of type 2 diabetes, and smoking status. In our models of exclusive duration, we further adjusted for duration of breastfeeding after introduction of formula or complementary foods. For women who are exclusively breastfeeding, the metabolic load of lactation may offset increased caloric intake, whereas for women who are partially breastfeeding, increased appetite may not be offset by caloric needs of breastfeeding. Thus, the net effect of breastfeeding on metabolism may be different for a woman who breastfeeds exclusively for 6 months and then weans completely compared with a woman who breastfeeds exclusively for 6 months and then continues to breastfeed partially.

We also used linear regression to quantify the association between adipokines and continuously measured duration of total and exclusive breastfeeding. To allow for nonlinear associations, we tested three models: linear duration; linear duration plus duration squared; and duration modeled with a three-knot quadratic spline. We used the likelihood ratio test to determine whether the more complex approaches improved the fit of the model, retaining the more complex model for likelihood ratio test $P < 0.05$. We present graphs of the model with the best fit for each adipokine to facilitate interpretation. To determine whether maternal BMI mediates the observed associations, we further adjusted for BMI at 3 years postpartum.

To test whether hormonal contraception use confounded observed associations, we further adjusted for 3-year postpartum use or nonuse among women for whom this data were available (n = 360 for the full cohort and n = 121 for fasting outcomes). Hormonal contraception use did not alter the observed associations, and it was therefore excluded from our analyses.

Both obesity and diabetes are risk factors for delayed lactogenesis and early weaning, so curtailed lactation may be a marker for an adverse metabolic profile. We therefore hypothesized that early weaning would be associated with higher leptin and lower adiponectin, PYY, and ghrelin levels. To test this hypothesis, we compared adipokine levels among mothers in four categories: *1*) those who had never breastfed; *2*) those who breastfed for shorter than they had planned and identified low milk supply as a reason for weaning or supplementing; *3*) those who breastfed for shorter than they had planned but did not identify milk supply problems as a reason for weaning or supplementing; and *4*) those who breastfed at least as long as they intended.

RESULTS

Breastfeeding was common in our cohort, with 88% reporting having ever breastfed, 26% breastfeeding for ≥12 months, and 42% breastfeeding exclusively for ≥3 months. Women who breastfed for ≥12 months were older and were more likely to have three or more children and less likely to be current smokers than those who breastfed for shorter periods (Table 1). Prepregnancy BMI was lower among women who would later initiate and sustain breastfeeding: women who did not breastfeed had a means (SD) BMI of 26.8 (6.0) kg/m^2, whereas those who would breastfeed for ≥12 months had a mean BMI of 23.7 (3.8) kg/m^2. Gestational weight gain, gestational glucose tolerance, and parental history of diabetes were not significantly associated with breastfeeding duration.

We found that leptin levels were inversely correlated with adiponectin and ghrelin (Spearman r = −0.32 and −0.40, respectively; $P < 0.0001$). Ghrelin was directly associated with adiponectin (r = 0.16, P = 0.03) and with PYY (r = 0.15, P = 0.047).

In unadjusted analyses, we found that longer breastfeeding duration was associated with lower leptin and higher ghrelin levels (Table 2). This association did not persist after adjustment for prepregnancy BMI and other risk factors (leptin: mean predicted value 7.6 ng/mL for ≥12 months of lactation vs. 8.9 ng/mL for no lactation, P = 0.45; ghrelin: 810.5 pg/mL, for ≥12 months of lactation vs.

TABLE 1
Baseline characteristics of study population by total duration of lactation. Data from 570 participants in Project Viva who presented for follow-up at 3 years postpartum without an intervening birth

	Months of lactation										
	None		>0 to <3		3 to <6		6 to <12		≥12		
	n = 70		n = 92		n = 117		n = 141		n = 150		
	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	P*
Prepregnancy BMI (kg/m^2)	26.8	(6.0)	26.9	(7.3)	24.9	(5.0)	24.8	(5.2)	23.7	(3.8)	<0.001
Gestational weight gain (kg)	15.1	(6.7)	14.6	(6.2)	15.7	(5.5)	14.5	(5.2)	14.9	(5.0)	0.54
Postpartum weight retention at 3 years (kg)	3.7	(7.5)	1.8	(10.0)	2.9	(5.7)	2.4	(5.6)	1.8	(5.7)	0.28
Age at 3-year visit	36.0	(4.8)	36.8	(6.0)	37.2	(5.3)	38.2	(4.6)	38.8	(5.0)	<0.001
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	
Race											0.10
Asian	0	(0)	5	(5)	7	(6)	5	(4)	5	(3)	
Black	12	(17)	16	(17)	21	(18)	13	(9)	19	(13)	
Hispanic	2	(3)	9	(10)	11	(9)	8	(6)	7	(5)	
Other	3	(4)	4	(4)	8	(7)	3	(2)	6	(4)	
White	53	(76)	58	(63)	70	(60)	112	(79)	113	(75)	
Parity											0.006
1	12	(17)	39	(42)	37	(32)	33	(23)	41	(27)	
2	36	(51)	40	(44)	57	(49)	73	(52)	64	(43)	
≥3	22	(31)	13	(14)	23	(20)	35	(25)	45	(30)	
Gestational glucose tolerance											0.74
Gestational diabetes	3	(4)	6	(7)	4	(3)	4	(3)	5	(3)	
Impaired glucose tolerance	3	(4)	2	(2)	8	(7)	5	(4)	6	(4)	
Transient hyperglycemia	5	(7)	12	(13)	13	(11)	14	(10)	11	(7)	
Normal	59	(84)	72	(78)	92	(79)	118	(84)	128	(85)	
Parental history of diabetes											0.85
Yes	11	(16)	17	(19)	19	(16)	20	(14)	20	(13)	
No	59	(84)	75	(82)	98	(84)	121	(86)	130	(87)	
Hormonal contraception†											0.04
Yes	4	(10)	16	(28)	7	(10)	17	(21)	16	(15)	
No	37	(90)	42	(72)	64	(90)	65	(79)	92	(85)	
Current smoker											<0.001
Yes	8	(11)	13	(14)	7	(6)	7	(5)	3	(2)	
No	50	(71)	73	(79)	104	(89)	129	(91)	139	(93)	
Missing	12	(17)	6	(7)	6	(5)	5	(4)	8	(5)	
Plan to exclusively breastfeed at 3 months ‡											<0.001
Yes	1	(1)	12	(13)	26	(22)	74	(52)	100	(67)	
No	69	(99)	80	(87)	91	(78)	67	(48)	50	(33)	
Fasting sample											0.34
Yes	16	(23)	30	(33)	40	(34)	38	(27)	51	(34)	
No	54	(77)	62	(67)	77	(66)	103	(73)	99	(66)	

*ANOVA P value for continuous variables, χ^2 P value for categorical variables. †Using hormonal contraception at 2–3 years postpartum, as reported at the 6-year follow-up visit. Missing for 210 participants. ‡At the 28-week interview, each participant reported how she intended to breastfeed her infant at 3 months postpartum.

714.8 pg/mL for no lactation, P = 0.32). We found no association between duration category and adiponectin or PYY.

When we measured the association between adipokine levels and total breastfeeding duration modeled as a continuous variable, we found lower leptin and higher ghrelin and PYY levels among women who had breastfed longer in unadjusted models (Fig. 1*A*, *E*, and *G*). After adjustment for prepregnancy BMI and other risk factors, this association persisted for ghrelin and PYY (Fig. 1*F* and *H*) (ghrelin predicted mean: 749.5 for none vs. 852.9 pg/mL for ≥12 months of breastfeeding, P = 0.05; PYY predicted geometric mean: 55.0 for none vs. 63.4 pg/mL for ≥12 months of breastfeeding, P = 0.03). We found no association with leptin in the multivariate models (Fig. 1*B*). We found a nonlinear association between adiponectin and duration of total breastfeeding in a multivariate-adjusted three-knot quadratic spline model (Fig. 1*D*) (likelihood ratio test, P = 0.04).

To determine whether breastfeeding intensity was associated with maternal metabolic markers at 3 years postpartum, we compared adipokine levels among women with different exclusive breastfeeding durations. In unadjusted models, we found higher leptin and lower ghrelin levels among women who had never exclusively breastfed (Table 3). When we adjusted for prepregnancy BMI and other risk factors, we found that differences in ghrelin levels were somewhat attenuated, but the association of exclusive breastfeeding with leptin was strengthened (leptin: multivariate adjusted mean predicted value for ≥6

TABLE 2
Adipokines at 3 years postpartum, by breastfeeding duration category: predicted values* from linear regression models

		Months of lactation							
		None		>0 to <3		3 to <6	6 to <12	≥12	
	n	Mean (95% CI)		Mean (95% CI)		Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	*P*†
Leptin (ng/mL) (*n*)		69		92		116	138	149	
Unadjusted	564	11.2 (9.7–12.6)		10.3 (9.0–11.5)		8.9 (7.8–10.0)	8.3 (7.3–9.4)	7.6 (6.6–8.6)	0.0002
Multivariable adjusted	564	8.9 (7.6–10.1)		8.0 (6.8–9.1)		7.8 (6.7–8.9)	7.8 (6.8–8.8)	7.8 (6.7–8.8)	0.45
Adiponectin (μg/mL) (*n*)		70		92		116	139	149	
Unadjusted	566	19.0 (17.0–21.0)		20.1 (18.4–21.9)		20.9 (19.4–22.5)	20.0 (18.6– 21.5)	20.2 (18.8–21.6)	0.70
Multivariable adjusted	566	21.2 (18.8–23.5)		22.9 (20.6–25.1)		22.8 (20.7–24.9)	20.9 (19.1–22.7)	20.7 (18.8–22.6)	0.11
Ghrelin (pg/mL) (*n*)		17		30		39	40	51	
Unadjusted	177	574.7 (429.8–719.5)		663.8 (554.8–772.8)		704.1 (608.4–799.7)	794.0 (699.5–888.4)	819.7 (736.0–903.3)	0.02
Multivariable adjusted	177	714.8 (557.3–872.3)		730.2 (595.0–865.3)		779.9 (669.1–890.7)	845.8 (745.2–946.5)	810.5 (696.3–924.6)	0.32
PYY (pg/mL) (*n*)‡		17		30		39	40	51	
Unadjusted	177	53.4 (45.8–62.3)		56.1 (50.0–63.0)		56.0 (50.6–62.0)	63.1 (57.1–69.7)	61.0 (55.8–66.7)	0.24
Multivariable adjusted	177	51.5 (42.4–62.7)		56.2 (47.5–66.4)		56.5 (49.1–65.0)	63.0 (55.6–71.3)	60.0 (52.1–69.1)	0.24

Data from 570 participants in Project Viva who presented for follow-up at 3 years postpartum without an intervening birth. *For the multivariate adjusted model, data presented are mean predicted values for a participant with a prepregnancy BMI of 25.1 kg/m^2, the mean for the study population, modeled using a three-knot quadratic spline model for BMI. The participant was a white woman aged 35–40 years, has two children, has no parental history of diabetes, had normal glucose tolerance, is a nonsmoker, and gained 15 kg during the index pregnancy. †Partial *F* test *P* values for differences among categories. ‡Results presented for PYY are geometric means. Because this outcome was not normally distributed, it was modeled on the log scale. Predicted values are exponentiated for presentation to improve interpretability.

months of exclusive lactation 9.0 vs. 11.4 ng/mL for no exclusive lactation, partial F P = 0.006). In the multivariate model, an association of duration of exclusive lactation with adiponectin levels became apparent. (adiponectin multivariate-adjusted mean for ≥6 months 25.9 vs. 20.4 μg/mL for no exclusive lactation, P = 0.05).

When we modeled duration of exclusive breastfeeding as a continuous variable, we found that longer durations were associated with lower leptin and higher ghrelin and PYY levels in unadjusted models (Figs. 2*A*, *E*, and *G*). In adjusted models, the direct association between duration of exclusive breastfeeding and ghrelin persisted (Fig. 2*F*) (predicted mean for never exclusively breastfeeding 790.6 vs. 1,008.1 pg/mL for ≥6 months of exclusive breastfeeding, $P < 0.01$), but the associations between exclusive breastfeeding and both PYY and leptin were no longer present (Fig. 2*B* and *H*). We found evidence of a nonlinear association between adiponectin and duration of exclusive breastfeeding in our multivariate-adjusted, three-knot quadratic spline model (Fig. 2*D*) (likelihood ratio test P = 0.03). Additional adjustment for maternal BMI at 3 years postpartum did not materially alter any observed associations between total or exclusive breastfeeding and adipokine levels.

To test our hypothesis that failed lactation would be associated with higher leptin and lower ghrelin, adiponectin, and PYY levels at 3 years postpartum, we created a variable for curtailed lactation, either as a result of low milk supply or issues not related to milk supply (Table 4). In our multivariate-adjusted models, we found the lowest leptin levels among women with a history of curtailed lactation and low supply (predicted mean 6.8 ng/mL vs. 7.5 ng/mL for curtailed lactation without supply issues, 8.0 ng/mL for successful breastfeeders, and 8.9 ng/mL for mothers who did not breastfeed, partial F test P = 0.05; P for comparisons between curtailed breastfeeding with supply problems versus both successful breastfeeding and never breastfeeding <0.05). Predicted mean levels of ghrelin were highest among women who breastfed successfully (824.4 pg/mL), followed by those with curtailed breastfeeding and supply problems (774.0 pg/mL), those who never breastfed (721.8 pg/mL), and curtailed breastfeeding without supply problems (713.4 pg/mL). We did not find any pattern of association between curtailed versus successful breastfeeding and adiponectin or PYY.

DISCUSSION

Consistent with our hypothesis, we found that long duration of both total and exclusive breastfeeding was associated with higher maternal ghrelin and pancreatic PYY levels at 3 years postpartum in a prospective cohort study, independent of other risk factors for metabolic disease. In contrast, we did not find that breastfeeding duration was independently associated with leptin levels, and there was no linear association of lactation with adiponectin. We further found no evidence that curtailed breastfeeding was associated with an adverse adipokine profile, suggesting that physiologic difficulties with lactation that might result in premature weaning are not themselves associated with maternal adipokine profile. This is the first study, to our knowledge, to measure the association between lactation and adipokine levels after weaning. Our results provide tentative evidence that longer lactation is associated with favorable changes in appetite regulation pathways that persist after weaning.

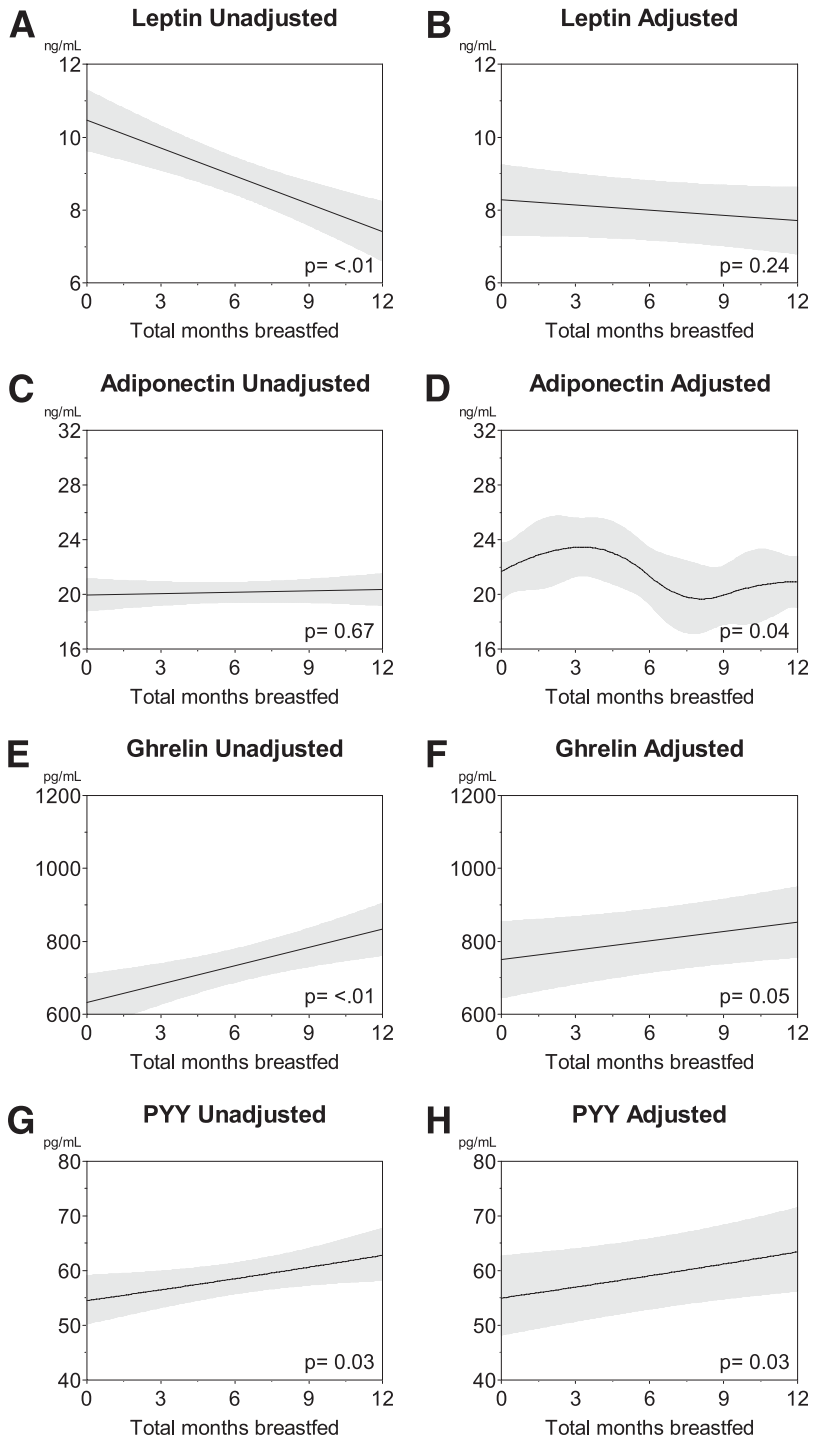

FIG. 1. Total duration of breastfeeding and adipokine levels at 3 years postpartum. The graphs show predicted means and 95% CIs (unadjusted as well as adjusted for pregravid BMI, race, age, parity, gestational weight gain and glucose tolerance, and parental history of type 2 diabetes) for a participant with a prepregnancy BMI of 25.1 kg/m^2, the mean for the study population, modeled using a three-knot quadratic spline model for BMI. The participant was a white woman, aged 35–40 years, has two children, has no parental history of diabetes, had normal glucose tolerance, is a nonsmoker, and gained 15 kg during the index pregnancy. *P* value is for likelihood ratio test for the presented model versus the model without breastfeeding duration.

Our results confirm and extend earlier work relating breastfeeding to differences in maternal metabolic outcomes after weaning. In large epidemiologic studies, we and others have reported associations between longer duration of lactation and reduced risk of diabetes (1–3), hypertension (8), higher HDL cholesterol levels postweaning adjusted for preconception levels (5), metabolic syndrome (6,7), and cardiovascular disease (3,9,27). The mechanism underlying this association is unknown. Lactation appears to mobilize adipose tissue accrued during pregnancy, and we have previously hypothesized that breastfeeding "resets" maternal metabolism after pregnancy, reducing disease risk (10).

In this study, we did not find any association between lactation duration and maternal leptin levels, once we adjusted for pregravid BMI. It is likely that unadjusted

TABLE 3
Adipokines at 3 years postpartum, by exclusive breastfeeding duration: predicted values* from linear regression models

		Months of exclusive lactation							
		None	>0 to <1		1 to <3		3 to <6	≥6	
	n	Mean (95% CI)	Mean (95% CI)		Mean (95% CI)		Mean (95% CI)	Mean (95% CI)	*P*†
Leptin (ng/mL) (*n*)		55	121		81		151	30	
Unadjusted	438	12.8 (10.2–15.3)	10.2 (8.9–11.6)		8.8 (7.2–10.4)		9.0 (7.4–10.6)	9.3 (6.5–12.1)	0.03
Multivariable adjusted	438	11.4 (9.5–13.3)	8.8 (7.5–10.1)		7.8 (6.4–9.2)		8.5 (7.1– 9.8)	9.0 (6.9–11.1)	0.006
Adiponectin (μg/mL) (*n*)		56	120		81		153	30	
Unadjusted	440	19.8 (16.2–23.4)	21.5 (19.6–23.5)		21.4 (19.1–23.7)		20.7 (18.4–22.9)	24.5 (20.6–28.5)	0.26
Multivariable adjusted	440	20.4 (16.9–24.0)	23.6 (21.1–26.1)		23.3 (20.7–25.9)		21.9 (19.4–24.4)	25.9 (21.9–29.9)	0.05
Ghrelin (pg/mL) (*n*)		11	39		22		50	14	
Unadjusted	136	665.1 (422.9–907.4)	721.5 (602.2–840.9)		728.7 (585.1–872.2)		794.6 (646.6–942.6)	1,034 (811.9–1,257)	0.05
Multivariable adjusted	136	706.3 (484.8–927.8)	754.4 (617.9–890.9)		865.0 (723.1–1,007)		810.0 (667.9–952.1)	920.9 (710.5–1,131)	0.2
PYY (pg/mL) (*n*)‡		11	39		21		50	14	
Unadjusted	135	61.0 (47.1–78.9)	55.5 (48.8–63.0)		56.9 (48.6–66.6)		62.0 (52.9–72.6)	53.5 (42.2–67.9)	0.46
Multivariable adjusted	135	57.0 (42.6–76.2)	56.7 (47.4–67.9)		56.4 (46.5–68.4)		60.2 (50.0–72.6)	51.2 (38.8–67.4)	0.63

Data from 570 participants in Project Viva who presented for follow-up at 3 years postpartum without an intervening birth. *For the multivariate-adjusted model, data presented are mean predicted values for a participant with a prepregnancy BMI of 25.1 kg/m^2, the mean for the study population, modeled using a three-knot quadratic spline model for BMI. This participant continued to breastfeed for up to 3 months after introducing formula or solid foods and is a white woman aged 35–40 years, has two children, has no parental history of diabetes, had normal glucose tolerance, is a nonsmoker, and gained 15 kg during the index pregnancy. †Partial *F* test *P* values for differences among categories. ‡Results presented for PYY are geometric means. Because this outcome was not normally distributed, it was modeled on the log scale. Predicted values are exponentiated for presentation to improve interpretability.

associations between breastfeeding duration and leptin are confounded by maternal BMI. We also found no consistent association between lactation duration and adiponectin levels. Because leptin and adiponectin are adipocyte products, these results do not support the hypothesis that changes in adipose tissue endocrinology mediate associations between breastfeeding and maternal metabolic outcomes.

We found a modest direct association between breastfeeding duration and levels of ghrelin and PYY, two gut-secreted peptide hormones that regulate appetite through reciprocal effects on hypothalamic orexogenic and anorexigenic pathways (28). In the fasting state, low PYY levels and high ghrelin levels stimulate hunger, whereas after feeding, PYY levels rise and ghrelin levels fall. Paradoxically, low ghrelin levels are associated with obesity, insulin resistance, hypertension, and type 2 diabetes in cross-sectional studies (13,14,16). In a small study (n = 18) of women at 4–5 weeks postpartum, Larson-Meyer et al. (29) found slightly higher fasting levels of ghrelin in lactating versus nonlactating mothers (971.8 [208.9] vs. 798.8 [271.8] pg/mL), but this difference was not statistically significant. PYY did not differ between lactating and nonlactating women. Among five women in that study who exclusively breastfed, ghrelin levels did not change from baseline to 24 weeks postpartum, despite reductions in total body fat and BMI (29). Ilcol et al. (30) measured ghrelin concentrations during lactation in 16 women and found increases in total ghrelin from 0–3 to 4–14 days postpartum, followed by slightly lower but stable levels from 15 to 30 days postpartum. The same authors measured total ghrelin in a cross-section sample of 159 women and found stable levels from 15 to 180 days postpartum.

Rodent studies (31,32) provide conflicting evidence regarding ghrelin's role in energy balance during lactation; however, cows bred for milk production have higher plasma ghrelin levels and greater energy intake during lactation (33). It is possible that long-term lactation and the associated negative energy balance induce changes in fasting ghrelin levels that persist after weaning, reducing the risk for metabolic disease. Alternately, women with higher baseline ghrelin levels may produce more milk, allowing them to continue breastfeeding longer. In this case, long breastfeeding durations would be a marker for reduced maternal metabolic risk.

Pancreatic PYY is an appetite-inhibiting peptide hormone secreted by the intestine in response to a meal (22). Obese individuals secrete less PYY than normal-weight individuals, suggesting an impaired response to satiety cues. Unlike the leptin resistance that can develop with obesity, overweight individuals retain a normal appetite response to infused PYY. We found a direct association between the duration of lactation and fasting PYY levels. This difference could improve energy balance, leading to more weight loss or less weight gain among women with longer durations of lactation. In support of this speculation, we previously found that women in our cohort with ≥6 months of exclusive breastfeeding had a lower BMI and reduced pregnancy-associated weight retention at 3 years, compared with women who had never breastfed exclusively (34).

When we measured the association between adipokine levels and curtailed breastfeeding, we found lower leptin and higher ghrelin levels among women who reported problems with milk supply, compared with women with curtailed breastfeeding who did not report supply

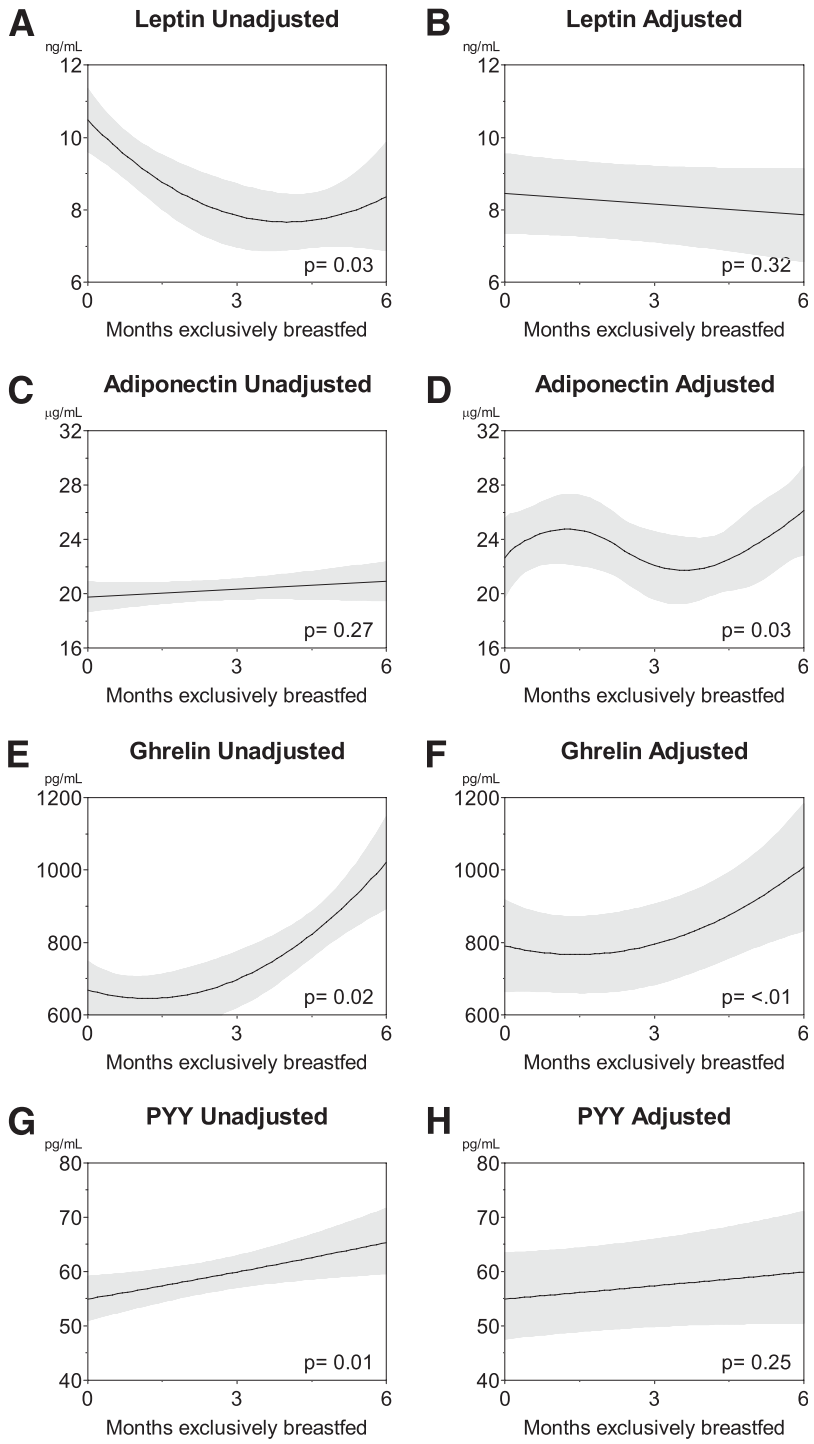

FIG. 2. Duration of exclusive breastfeeding and adipokine levels at 3 years postpartum. The graphs show predicted means and 95% CIs (unadjusted as well as adjusted for pregravid BMI, race, age, parity, gestational weight gain and glucose tolerance, and parental history of type 2 diabetes) for a participant with a prepregnancy BMI of 25.1 kg/m^2, the mean for the study population, modeled using a three-knot quadratic spline model for BMI. This participant continued to breastfeed for up to 3 months after introducing formula or solid foods, is a white woman aged 35–40 years, has two children, has no parental history of diabetes, had normal glucose tolerance, is a nonsmoker, and gained 15 kg during the index pregnancy. *P* value is for likelihood ratio test for the presented model versus the model without breastfeeding duration.

problems. We acknowledge that we had only limited ability to capture biologic versus cultural or social reasons for weaning; however, our results do not support the hypothesis that difficulty with milk production is a marker for an adverse adipokine profile.

No other studies to our knowledge have measured associations between the duration of breastfeeding and ghrelin or PYY levels after weaning; however, there is tentative evidence that lactation is associated with long-term changes in other hypothalamic neuroendocrine pathways. During lactation, breastfeeding women have reduced autonomic and adrenal responses to stressors, compared with nonlactating postpartum control subjects (35–39). Differences in hypothalamic-pituitary-adrenal activity may

TABLE 4
Adipokine levels at 3 years postpartum, comparing women who never breastfed, whose breastfeeding was curtailed, or who breastfed successfully:* predicted values† from linear regression models

	n	Never breastfed	Curtailed breastfeeding, problems with milk supply*	Curtailed breastfeeding, no problems with milk supply*	Successful breastfeeding*	*P*‡
Leptin (ng/mL) (*n*)		69	67	45	383	
Unadjusted	564	11.2 (9.7–12.6)§	7.9 (6.4–9.3)	9.0 (7.2–10.8)	8.7 (8.1–9.3)	<0.01
Multivariable adjusted	564	8.9 (7.6–10.1)§	6.8 (5.6–8.1)	7.5 (6.0–9.0)	8.0 (7.1–8.8)§	0.05
Adiponectin (μg/mL) (*n*)		70	67	45	384	
Unadjusted	566	19.0 (17.0–21.0)	21.3 (19.2–23.3)	19.9 (17.4–22.4)	20.2 (19.4–21.1)	0.50
Multivariable adjusted	566	20.9 (18.6–23.3)	22.7 (20.3–25.1)	21.3 (18.5–24.1)	21.2 (19.7–22.8)	0.58
Ghrelin (pg/mL) (*n*)		17	23	20	117	
Unadjusted	177	574.7 (427.9–721.5)‖	718.9 (592.7–845.1)	682.2 (546.8–817.5)	775.7 (719.8–831.6)	0.07
Multivariable adjusted	177	721.8 (566.6–877.1)	774.0 (645.4–902.5)	713.4 (568.5–858.3)	824.4 (736.3–912.4)	0.18
PYY (pg/mL) (*n*)¶		17	23	21	116	
Unadjusted	177	53.4 (45.7–62.4)	57.5 (50.4–65.7)	56.5 (49.2–65.0)	60.2 (56.7–63.9)	0.48
Multivariable adjusted	177	52.3 (43.0–63.5)	57.0 (48.5–67.0)	58.8 (49.1–70.3)	60.9 (54.5–68.0)	0.33

Data from 570 participants in Project Viva who presented for follow-up at 3 years postpartum without an intervening birth. *We used data on mothers' intended duration of breastfeeding, as reported at 28 weeks of pregnancy to define curtailed breastfeeding as either *1*) weaning prior to 3 months among women who planned to breastfeed for more than 3 months or *2*) introduction of formula prior to 3 months among women who planned to breastfeed exclusively for at least 3 months. At the 6-month interview, mothers who had weaned or were supplementing identified reasons for introducing formula or weaning from a structured list. We classified women as having problems with milk supply if they endorsed any of the following: "I wasn't producing enough breast milk to satisfy my baby," "I had difficulty or didn't like pumping breast milk," or "My baby was not gaining enough weight with breastfeeding." †For the multivariate-adjusted model, data presented are mean predicted values for a participant with a prepregnancy BMI of 25.1 kg/m^2, the mean for the study population, modeled using a three-knot quadratic spline model for BMI. This participant was a white woman aged 35–40 years, has two children, has no parental history of diabetes, had normal glucose tolerance, is a nonsmoker, and gained 15 kg during the index pregnancy. ‡Partial *F* test *P* values for differences among categories. §$P < 0.05$ vs. curtailed breastfeeding problems with supply group. ‖$P < 0.05$, vs. successful breastfeeding group. ¶Results presented for PYY are geometric means. Because this outcome was not normally distributed, it was modeled on the log scale. Predicted values are exponentiated for presentation to improve interpretability.

persist after weaning: Lankarani-Fard (40) measured fasting cortisol levels among 749 postmenopausal women and reported higher levels among women with >12 months of lifetime lactation, compared with women with shorter durations of breastfeeding. Coupled with our finding of higher fasting ghrelin and PYY levels among women with longer lactation, these results suggest that lactation may have lasting effects on central neuroendocrine pathways.

Strengths of our study include its prospective assessment of maternal BMI, metabolic disease risk factors, and breastfeeding intention and duration; measurement of exclusive breastfeeding; measurement of multiple adipokines at 3 years; and our very high rate of breastfeeding initiation and continuation. We also faced several limitations, including the relatively small number of participants with fasting blood samples, which may have limited our power to detect subtle differences among duration categories. To maximize our power, we therefore modeled associations of adipokines with continuous as well as categorical measures of lactation duration.

Our study's cross-sectional design also limits interpretation of results. Without prepregnancy measures of metabolic markers, we cannot determine whether lactation causes favorable changes in peptide hormone levels or whether more favorable profiles predispose to successful breastfeeding. Moreover, levels of measured adipokines are correlated, and our findings for PYY and ghrelin likely reflect interdependent mechanisms. In a cross-sectional, observational study such as ours, we cannot determine the causal direction of associations among adipokines. Nevertheless, our study is the first, to our knowledge, to measure adipokines after weaning, and our results provide evidence to support future, longitudinal studies. We also had limited information on hormonal contraception use at the time of the 3-year visit. However, adjustment for contraception among those for whom data were available did not alter our results, reducing the likelihood that more complete information would change our results. We also did not measure fat mass at the time of blood sample collection. Finally, false-positives are a concern, because we measured four different adipokines and performed multiple tests of association. However, our measures reflect specific hypotheses regarding pathways that are not independent, so statistical correction for multiple outcomes would be overly conservative.

In conclusion, in a prospective study of maternal and infant health, we found that longer duration of breastfeeding was associated with higher maternal levels of ghrelin and PYY at 3 years postpartum. These two gut peptides regulate appetite and are associated with reduced risk of metabolic disease. Our findings provide tentative evidence that changes in hypothalamic appetite regulation may mediate associations between longer lactation and reduced risk of maternal metabolic disease. Additional studies will be needed to confirm these findings in other populations and to determine whether these associations are causal.

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A.M.S. analyzed the data and wrote the manuscript. C.M. completed the laboratory assays, contributed to the

discussion, and reviewed and edited the manuscript. K.K. provided advice on statistical methods and reviewed and edited the manuscript. S.R.-S. analyzed the data and reviewed and edited the manuscript. E.P.G. contributed to the discussion and reviewed and edited the manuscript. M.W.G. and J.R.-E. researched the data, contributed to the discussion, and reviewed and edited the manuscript.

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