Relationship of Postpartum Levels of Cystatin and High-Sensitivity C-Reactive Protein and Duration of Lactation in Mothers with Previous Gestational Hypertension or Preeclampsia

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Abstract

Background: Women with hypertensive disorders of pregnancy are at increased risk of cardiovascular disease in later life. We sought to determine the association between lactation and markers of maternal cardiovascular health among postpartum women with and without hypertensive disorders of pregnancy via measures of inflammation (high-sensitivity C-reactive protein [hsCRP]) and renal function (cystatin C).

Materials and Methods: This prospective cohort study enrolled primarily overweight and obese women during early pregnancy. At a postpartum study visit occurring 6–24 months after delivery, we collected data on lactation duration and measured hsCRP and cystatin C. We assessed associations between lactation duration and levels of hsCRP and cystatin C among normotensive women and women with preeclampsia or gestational hypertension using analysis of variance and chi-squared tests. Linear regression models adjusted for age, race, education, prepregnancy body mass index, current smoking, and time since delivery.

Results: Of 425 women, 37 (9%) had preeclampsia and 48 (11%) had gestational hypertension during enrollment pregnancy. The postpartum visit occurred at a mean of 8.6 ± 4.4 months after delivery. Women with a history of preeclampsia had significantly higher levels of cystatin C (mean 0.86 versus 0.78 mg/L; $p=0.03$) compared with normotensive women, but nonsignificant elevation in hsCRP (mean 8.39 versus 6.04 mg/L; $p=0.08$). Women with gestational hypertension had no differences in mean hsCRP or cystatin C compared with normotensive women. Among the 237 women with any lactation, 78 (18%) lactated for at least 6 months. Lactation duration both in the overall sample and among women with gestational hypertension or preeclampsia was not associated with levels of hsCRP or cystatin C.

Conclusions: Preeclampsia history was associated with elevated postpartum levels of cystatin C; however, duration of lactation was not associated with postpartum hsCRP or cystatin C, regardless of history of gestational hypertension or preeclampsia. Further research is needed on mechanisms through which lactation may affect maternal risk of cardiovascular disease.

Keywords: cystatin C, gestational hypertension, high-sensitivity C-reactive protein, lactation, preeclampsia

Introduction

THE INCIDENCES OF PREECLAMPSIA and gestational hypertension are increasing among pregnant women in the United States.^{1–4} This trend is concerning, because these hypertensive disorders of pregnancy are known risk factors

for development of maternal cardiovascular disease (CVD) in later life. The physiologic mechanisms by which hypertensive disorders of pregnancy predispose women to CVD are currently unknown. While pregnancy and lactation involve adaptations in maternal immune regulation, volume status, and renal function, for women who develop hypertensive

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disorders of pregnancy, these processes are compromised. This dysfunction is manifested in abnormalities of markers of inflammation and renal function, such as high-sensitivity C-reactive protein (hsCRP)^{5–8} and cystatin C,⁹ respectively.

Lactation may be one way to counteract the cardiovascular risks associated with hypertensive disorders of pregnancy.¹⁰ A dose-dependent inverse relationship between duration of lactation and development of maternal subclinical and clinical CVD has previously been reported.^{11–13} However, the association of lactation and biomarkers of cardiovascular risk, such as hsCRP and cystatin C, is unknown.

hsCRP is a marker of inflammation and has been associated with increased risk of CVD and heart failure.^{14–17} hsCRP has now been incorporated into risk estimators for cardiovascular events, including the Reynolds Risk Score, which has been suggested to be superior to the Framingham Risk Score for global cardiovascular risk prediction, especially in women.^{18,19} Modifiable health behaviors such as weight loss²⁰ and exercise²¹ have been correlated with decreasing hsCRP levels. One study has also suggested that mothers who are lactating have lower hsCRP levels than women who do not lactate, although this difference was not statistically significant.²²

Cystatin C is a protease inhibitor that is a marker of renal function and has been used to estimate glomerular filtration rate; it has also been associated with CVD and cardiovascular mortality.^{23–26} Among women with preeclampsia, maternal levels of cystatin C are elevated.^{27,28} Thus, cystatin C has been suggested as a possible diagnostic marker for preeclampsia,^{27,29} which when combined with other predictive markers is useful in the first trimester.³⁰ Postpartum cystatin C levels in relation to lactation have not previously been studied. In this study, we characterize the relationships between postpartum levels of hsCRP and cystatin C, by duration of lactation, and history of hypertensive disorders of pregnancy.

Materials and Methods

Participants

The study population was derived from a cohort of women enrolled in the Prenatal Exposures and Preeclampsia Prevention (PEPP3) study, a prospective study designed to evaluate the impact of obesity on preeclampsia in women who receive antepartum, delivery, and postpartum care at the Magee Women's Hospital of the University of Pittsburgh Medical Center. Women eligible for PEPP3 were 18–40 years of age and 6–16 weeks pregnant with a single fetus. Exclusion criteria included body mass index (BMI) <18, pre-existing hypertension, diabetes, seizure disorders, collagen vascular disorder, drug or alcohol abuse, and liver, heart, or kidney disease. Women were also excluded from initial or further participation in the event of a major fetal anomaly or fetal demise. To examine mechanisms linking obesity to preeclampsia and gestational hypertension, overweight and obese women (BMI >25 kg/m²) were preferentially recruited to comprise 85% of the study population; a small group of lean women were enrolled for comparison.

As part of the study protocol, women were asked to attend a postpartum visit at least 3 months and up to 24 months after delivery. For this analysis, we included women attending a postpartum study follow-up visit who had not become pregnant in the interim and who completed both a blood draw and breastfeeding questionnaire at the postpartum visit. We

excluded women who had both a postpartum visit <6 months after delivery and who were still breastfeeding at that visit, as a determination could not yet be made regarding their duration of lactation. All women provided written informed consent, and this analysis received exempt approval by the University of Pittsburgh's Institutional Review Board.

Data collection

At the enrollment visit during pregnancy, participants completed a questionnaire that included demographic information (age, race, parity, marital status, education, income, occupation, smoking history, and plans for breastfeeding) as well as self-reported prepregnancy height and weight. The correlation between the first study weight measure and the self-reported prepregnancy weight was high (>0.97). Prepregnancy BMI (kg/m²) was calculated with self-reported weight and height and categorized based on the World Health Organization guidelines as normal weight (BMI 18.5–24.9), overweight (BMI 25–29.9), obese class I (BMI 30–34.9), class II (BMI 35–39.9), and class III (BMI ≥40).

Delivery data abstracted from the electronic medical records of participants included gestational age at delivery, delivery type, and pregnancy complications. Gestational hypertension (≥2 BP measurements >140/90) and preeclampsia (gestational hypertension plus proteinuria) were defined based on the American College of Obstetricians and Gynecologists (ACOG) guidelines in 2002³¹ and adjudicated by the PEPP3 research team based on chart reviews. Gestational diabetes was defined as a 1-hour 50 g glucose tolerance test of ≥135 mg/dL followed by a 3-hour 100 g glucose tolerance test with two or more values ≥95 mg/dL at 1 hour, 180 mg/dL at 2 hours, 155 mg/dL at 3 hours, or 140 mg/dL at 4 hours.

Lactation history

At the postpartum visit, participants completed a questionnaire that assessed their breastfeeding practices. To assess the duration of breastfeeding, participants were initially asked “Did you ever breastfeed or pump breast milk to feed your new baby after delivery, even for a short period of time?” Those who answered “yes” were asked two additional questions: “Are you currently breastfeeding or feeding pumped milk to your new baby?” and “How many weeks or months did you breastfeed or pump milk to feed your baby?” Based on these questions, participants were categorized into three groups: (1) never lactated, (2) lactated <6 months, and (3) lactated ≥6 months. A cut-off of lactation duration ≥6 months was chosen based on the American Academy of Pediatrics recommendation for exclusive breastfeeding duration.³² Women who lactated <1 week were placed in the never lactated category.

hsCRP and cystatin C measurements

Fasting blood samples were collected from the participants at their postpartum visit. hsCRP was measured using 100 µL of serum with reagents obtained from Beckman Coulter and analyzed on an Olympus AU400 Chemistry Analyzer (Olympus America, Inc). In this procedure, the CRP in the sample reacts with goat anti-CRP antibodies coated on latex particles. The increase in absorbance is measured turbidimetrically. Blanks, controls, and standards (0.5–20 mg/L) are run simultaneously

with all samples. The intra- and inter-assay coefficients of variation are 1.5% and 3.4%, respectively.

Cystatin C was also measured turbidimetrically on an Olympus AU 400 using 100 μ L of sample and reagents obtained from DakoCytomation N. America, Inc. Samples were incubated at room temperature for 5 minutes with rabbit anti-cystatin C antibodies coupled to polystyrene particles. The increase in absorbance at 540 nm was then measured. Blanks, controls, and standards (0.4–8.0 mg/L) were run simultaneously with all samples. The intra- and inter-assay coefficients of variation for this protocol are 1.7% and 2.2%, respectively.

Statistical analyses

Differences in maternal characteristics among women who attended the postpartum visit compared with those who did not were analyzed using analysis of variance (ANOVA) for continuous variables and chi-squared tests for categorical variables. Similarly, maternal characteristics were compared according to lactation history using ANOVA for continuous variables and chi-squared tests for categorical variables. We evaluated the relationship between postpartum levels of hsCRP and cystatin C and gestational hypertension or preeclampsia using unpaired *t*-tests. We also examined the relationship between postpartum levels of hsCRP and cystatin C and duration of lactation for women with (1) normotensive pregnancies, (2) preeclamptic pregnancies, and (3) gestational hypertensive pregnancies. We used Fisher's exact tests and chi-squared tests to compare differences in hsCRP as a categorical variable (moderately elevated: hsCRP ≥ 1.5 ; severely elevated: hsCRP ≥ 3 mg/L as suggested by prior studies^{33,34}) among the three lactation groups. We used ANOVA to compare differences in hsCRP as a continuous variable among the lactation groups. hsCRP data were analyzed without transformation to assist with clinical interpretation, but when replicated using log transformations, results were unchanged. Differences in cystatin C levels (mg/L) among the lactation groups were compared using ANOVA. Linear regression was used to adjust for potential confounding variables, including maternal education (the socioeconomic status indicator that has been most strongly related to pregnancy complications and to CVD risk in women^{35,36}), age, race, time since delivery, current smoking, and prepregnancy BMI. Potential confounders were identified as those that varied significantly by lactation category and those known to be related to hsCRP or cystatin C.

We performed sensitivity analyses restricting the sample to nulliparous women and then to obese women (BMI >30) to confirm that the observed relationships were consistent across these subgroups. Statistical analyses were conducted using Stata version 13.0.

Results

Participants

The initial PEPP3 cohort comprised 651 predominantly overweight or obese and African American women. Of these, 439 attended the postpartum visit and 425 were included in this study (mean postpartum visit at 8.6 months, standard deviation 4.4 months postpartum). Of those who were excluded, five did not have blood drawn, three did not document

breastfeeding duration, and six had a postpartum visit <6 months after delivery and were still breastfeeding. Women who attended the postpartum visit were more likely to be white, have higher socioeconomic status, insured, and primiparous compared with those who did not (Supplementary Table S1).

Duration of lactation differed by age, race, education status, smoking status, and income (Table 1). There were 37 women (9%) with preeclampsia and 48 (11%) with gestational hypertension; the prevalence of preeclampsia and gestational hypertension was similar among the lactation groups. Five women who attended a postpartum visit had a history of chronic hypertension; however, none of these women developed superimposed preeclampsia. Of the women who never lactated, 50 (28%) had planned to breastfeed before delivery.

Postpartum hsCRP and cystatin C by diagnosis of hypertensive disorder of pregnancy

Compared with women who remained normotensive throughout pregnancy, those with a history of preeclampsia had higher mean levels of postpartum cystatin C (0.86 versus 0.78 mg/L; $p=0.03$), but no significant difference in postpartum hsCRP (8.39 versus 6.04 mg/L; $p=0.08$). Women with a history of gestational hypertension had no differences in mean hsCRP or cystatin C levels compared with women who remained normotensive (7.28 versus 6.04 mg/L; $p=0.34$, and 0.76 versus 0.78 mg/L; $p=0.25$, respectively).

Postpartum cystatin C and hsCRP by lactation duration

Duration of lactation was not associated with cystatin C or hsCRP among normotensive women or those with gestational hypertension or preeclampsia (Tables 2–4). Sensitivity analyses including only women with BMI ≥ 30 , only primiparous women, or only women who had stopped breastfeeding by the time of our analyses did not change results.

Multivariable linear regression models

After adjusting for age, race, education, current smoking, time since delivery, and prepregnancy BMI, there were no differences in hsCRP levels among women who lactated <6 months or ≥ 6 months compared with women who never lactated, when stratified by hypertensive disorders of pregnancy (Table 5). In women with a history of preeclampsia, there was an association with higher cystatin C ($\beta=0.49$; CI=0.01–0.98) for those who lactated ≥ 6 months compared with those who never lactated with a trend toward higher cystatin C in fully adjusted models.

Discussion

In this prospective study that followed pregnant women for an average of 8 months postpartum, we found that the duration of lactation was not associated with postpartum levels of hsCRP or cystatin C among women who remained normotensive throughout pregnancy, nor among those who developed preeclampsia or gestational hypertension. Although women with a history of preeclampsia had elevated levels of postpartum cystatin C in this study, this association

TABLE 1. SAMPLE CHARACTERISTICS BY LACTATION GROUP AT BASELINE 6–16 WEEKS GESTATION (N=425)

	<i>Never lactated</i> (n=188)	<i>Lactated <6 months</i> (n=159)	<i>Lactated ≥6 months</i> (n=78)	p
Age, years, mean, SD	22.8, 4.2	24.1, 3.9	25.0, 4.2	<0.01
Race, n (%)				0.04
Black	139 (74)	100 (63)	44 (56)	
White	47 (25)	55 (35)	31 (40)	
Other	2 (1)	4 (3)	3 (4)	
Married, n (%)	56 (30)	66 (42)	35 (45)	0.02
Education, n (%)				<0.01
Less than HS	24 (13)	9 (6)	3 (4)	
HS or GED	117 (63)	71 (45)	27 (35)	
College or greater	46 (25)	78 (49)	48 (62)	
Income per year, n (%)				<0.01
<\$20,000	113 (60)	85 (54)	41 (53)	
\$20,000–\$49,999	29 (16)	40 (25)	16 (21)	
>\$50,000	5 (3)	10 (6)	12 (15)	
Unknown	40 (21)	23 (15)	9 (12)	
Insurance, n (%)				<0.01
Private	6 (3)	20 (13)	15 (20)	
Medicaid	136 (74)	89 (56)	35 (46)	
None at enrollment	39 (21)	50 (31)	26 (34)	
Smoking (current), n (%)	81 (43)	57 (36)	14 (18)	<0.01
Smoking (lifetime), n (%)	90 (48)	67 (42)	30 (38)	0.30
Nulliparous, n (%)	139 (74)	126 (79)	60 (77)	0.51
Parity, n (%)				0.24
0	139 (74)	126 (79)	60 (77)	
1	27 (14)	24 (15)	13 (17)	
≥2	22 (12)	9 (6)	5 (6)	
Prepregnancy BMI, mean, SD	32.7, 8.1	32.1, 7.1	31.5, 7.2	0.51
Prepregnancy BMI category, n (%)				0.81
Normal	31 (16)	21 (13)	14 (18)	
Overweight	40 (21)	39 (25)	22 (28)	
Class I obese	55 (29)	51 (32)	23 (29)	
Class II obese	32 (17)	28 (18)	9 (12)	
Class III obese	30 (16)	20 (13)	10 (13)	
Index pregnancy characteristics, n (%)				
Preterm birth (<37 weeks)	18 (10)	20 (13)	9 (12)	0.67
Preeclampsia	18 (10)	14 (9)	5 (6)	0.70
Gestational HTN	24 (13)	17 (11)	7 (9)	0.63
Gestational diabetes	2 (1)	8 (5)	3 (4)	0.09
Planned breastfeeding	50 (28)	124 (83)	72 (97)	<0.01
SBP at follow-up, mean, SD	112.5, 11.5	113.3, 10.3	113.7, 11.9	0.71
DBP at follow-up, mean, SD	71.4, 9.1	72.4, 8.3	72.0, 9.6	0.58
BMI at follow-up, mean, SD	34.7, 8.5	34.3, 7.6	33.4, 7.6	0.47

BMI, body mass index; DBP, diastolic blood pressure; GED, general educational development; HS, high school; HTN, hypertension; SBP, systolic blood pressure; SD, standard deviation.

TABLE 2. MEDIAN HIGH-SENSITIVITY C-REACTIVE PROTEIN (MG/L) BY LACTATION DURATION AND HYPERTENSIVE DISORDERS OF PREGNANCY

	<i>Never lactated</i> (n=188)	<i>Lactated <6 months</i> (n=159)	<i>Lactated ≥6 months</i> (n=78)	p
Normotensive, median (IQR)	3.7 (8.9)	3.2 (4.4)	3.3 (5.88)	0.70
Gestational HTN, median (IQR)	4.3 (7.0)	3.2 (3.9)	3.8 (5.7)	0.34
Preeclampsia, median (IQR)	5.2 (8.5)	5.7 (13)	2.4 (5.28)	0.67

IQR, interquartile range.

TABLE 3. PROPORTION OF WOMEN WITH ELEVATED HIGH-SENSITIVITY C-REACTIVE PROTEIN BY LACTATION DURATION AND HYPERTENSIVE DISORDERS OF PREGNANCY

	<i>Never lactated</i> (n = 188)	<i>Lactated <6 months</i> (n = 159)	<i>Lactated ≥6 months</i> (n = 78)	p
Normotensive, n (%)				0.99
hsCRP <1.5	40 (27.4)	36 (28.13)	18 (27.3)	
hsCRP 1.5–3.0	28 (19.2)	26 (20.3)	14 (21.2)	
hsCRP ≥3.0	78 (53.4)	66 (52.6)	34 (51.5)	
Gestational HTN, n (%)				0.26
hsCRP <1.5	6 (25.0)	4 (23.5)	1 (14.3)	
hsCRP 1.5–3.0	4 (16.7)	3 (17.7)	1 (14.3)	
hsCRP ≥3.0	14 (58.3)	10 (58.8)	5 (71.4)	
Preeclampsia, n (%)				1.0
hsCRP <1.5	3 (16.7)	4 (28.6)	1 (20.0)	
hsCRP 1.5–3.0	1 (5.6)	1 (7.1)	2 (40.0)	
hsCRP ≥3.0	14 (77.8)	9 (64.3)	2 (40.0)	

was not seen among women with a history of gestational hypertension.

hsCRP is an inflammatory marker that is known to be elevated during pregnancy. In a large prospective cohort study, longer duration of lactation was associated with lower levels of hsCRP 4–12 years after pregnancy among normotensive women; however, these results did not retain significance after adjusting for covariates.³⁷ An additional cohort study looked at shorter-term (average 3 years) follow-up and lactation association with hsCRP levels and again showed a nonsignificant reduction in hsCRP after adjusting for covariates.³⁸ In a small cohort study of 45 women, those who breastfed >5 months did not have a significant difference in hsCRP levels compared with those who did not breastfeed.³⁹ Our study builds on these prior studies by explicitly examining the differences in hsCRP according to hypertensive disorders of pregnancy; our findings indicate no difference in hsCRP levels by duration of lactation, irrespective of a history of gestational hypertension. Notably, obesity is known to be associated with elevations in inflammatory markers,⁴⁰ and an association between postpartum BMI and elevation in postpartum hsCRP levels has been suggested.⁴¹ Since the women in our study were preferentially recruited to be overweight or obese, any differences in hsCRP levels with longer duration of lactation may have been masked. We did not see a significant difference in our results with sensitivity analyses restricted to only obese or only normal-weight women; however, the small number of women in each group with these subanalyses limit the power to detect differences. It is also worth noting that, of the women who did not lactate in our study, a high percentage (43%) were current smokers at enrollment. Since hsCRP levels have been shown to be higher among active smokers,⁴² this may also have blunted

the lactation benefits seen in our results, although results were unchanged even after adjusting for current smoking status.

For cystatin C, we also saw no difference in postpartum levels by lactation duration. This is the first study to address the association of lactation with this renal functional marker. However, recent studies have shown an association with elevated cystatin C levels in early pregnancy and later development of preeclampsia, suggesting a role in the pathophysiologic process.⁴³

Lactation is known to be associated with an improved cardiometabolic risk profile; however, the exact mechanisms remain unknown.^{44,45} Our study adds to a growing body of literature addressing the possible underlying mechanisms. Our study ultimately suggests that the mechanisms through which lactation affects maternal health do not appear to involve hsCRP or cystatin C. However, it is important to note that this study had a relatively short follow-up period, with a mean of 8 months, which may not be long enough to appreciate an association of lactation with hsCRP or cystatin C levels. Additionally, the intensity of lactation may play a role in reducing postpartum insulin resistance⁴⁶ and should be assessed in future studies looking at inflammatory markers such as hsCRP.

This study had several additional limitations. Most importantly, the study included a relatively small number of participants, in particular for those with hypertensive disorders of pregnancy. Participants were evaluated at only one study site, which may limit the generalizability of the findings. With regard to the measurement of breastfeeding, participants were asked to recall their lactation duration by survey, which may be subject to recall bias. Although the majority of women in our study were primiparous, we did not assess lactation duration during previous pregnancies and thus were not able to include lifetime lactation duration in our

TABLE 4. MEAN CYSTATIN C (MG/L) BY LACTATION DURATION AND HYPERTENSIVE DISORDERS OF PREGNANCY

	<i>Never lactated</i> (n = 188)	<i>Lactated <6 months</i> (n = 159)	<i>Lactated ≥6 months</i> (n = 78)	p
Normotensive, mean (SD)	0.79 (0.10)	0.78 (0.19)	0.76 (0.11)	0.38
Gestational HTN, mean (SD)	0.76 (0.09)	0.74 (0.08)	0.79 (0.10)	0.55
Preeclampsia, mean (SD)	0.78 (0.14)	0.81 (0.10)	1.27 (1.35)	0.12

TABLE 5. MULTIVARIABLE MODELS OF THE RELATIONSHIP BETWEEN LACTATION AND HIGH-SENSITIVITY C-REACTIVE PROTEIN AND CYSTATIN C LEVELS STRATIFIED BY HYPERTENSIVE DISORDERS OF PREGNANCY

	hsCRP				Cystatin C			
	Unadjusted		Fully adjusted ^a		Unadjusted		Fully adjusted ^a	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Normotensive								
Lactated <6 months	-0.73	-2.58 to 1.12	-0.16	-1.97 to 1.66	-0.01	-0.05 to 0.02	-0.02	-0.05 to 0.02
Lactated $\geq$ 6 months	0.02	-2.24 to 2.29	1.45	-0.85 to 3.74	-0.03	-0.07 to 0.01	-0.03	-0.08 to 0.01
Gestational HTN								
Lactated <6 months	-5.26	-13.1 to 2.53	-2.50	-11.4 to 6.41	-0.02	-0.07 to 0.04	0.01	-0.06 to 0.07
Lactated $\geq$ 6 months	-5.07	-15.6 to 5.5	-0.85	-12.9 to 11.3	0.03	-0.05 to 0.10	0.04	-0.04 to 0.13
Preeclampsia								
Lactated <6 months	0.76	-5.67 to 7.19	1.19	-7.12 to 9.51	0.03	-0.31 to 0.38	0.03	-0.36 to 0.41
Lactated $\geq$ 6 months	-3.39	-12.5 to 5.73	-3.03	-15.2 to 9.13	0.49	0.01 to 0.98 ^b	0.53	-0.04 to 1.09

^aAdjusted for age, race, education, current smoking, time since delivery, and prepregnancy BMI with never lactated as reference category.

^bp-Value <0.05.

demographic description. Additionally, our lactation categories may not be refined enough to observe differences in inflammatory markers. It is possible that there is a more nuanced, graded effect of lactation intensity and duration on these markers, which our study did not account for. We adjusted for time since delivery; however, our results are limited by follow-up visits that vary in time since lactation cessation. Another study limitation is the criteria used to diagnose hypertensive disorders of pregnancy among women in our sample; we used the ACOG definitions of gestational hypertension and preeclampsia that were in place at the time of our study; however, these definitions have since been updated.⁴⁷ The definition for preeclampsia now includes women without proteinuria but have other systemic findings consistent with preeclampsia. With the newer definitions, some women we categorized as having gestational hypertension may have shifted to the preeclampsia group. Lastly, there were significant socioeconomic differences between women who lactated and those who did not. Although we adjusted for some measures of socioeconomic status, including education, concurrent adjustment for other socioeconomic variables was precluded by the small numbers of participants in each category.

Our study also had a number of strengths, including the recruitment of a diverse sample of women who were predominantly overweight or obese, of African American origin, and of lower socioeconomic status. These populations are at a higher risk of CVD and often remain underrepresented in clinical research. Another strength of our study design was the collection of lactation history in a standardized fashion within months of delivery (as opposed to retrospective research asking women to recall often years prior). In addition, detailed information on hypertensive disorders of pregnancy was collected from medical records using a formal research adjudication protocol, thus ensuring that preeclampsia and gestational hypertension were accurately distinguished.

Conclusion

Women with a history of preeclampsia have elevated levels of postpartum cystatin C compared with those who were

normotensive during pregnancy. Lactation does not appear to be associated with variation in the levels of hsCRP or cystatin C among overweight or obese women, whether or not they remained normotensive throughout pregnancy. Future studies with larger cohorts and longer follow-up duration are needed to further elucidate the relationships between hypertensive disorders of pregnancy, lactation, and markers of inflammation, renal function, and cardiovascular risk in later life.

Acknowledgment

The PEPP3 study was funded by NICHD (P01 HD 30367) and the University of Pittsburgh Clinical and Translational Research Center (UL1 TR000005).

Disclosure Statement

No competing financial interests exist.

Supplementary Material

Supplementary Table S1

References

- Wallis AB, Saftlas AF, Hsia J, et al. Secular trends in the rates of preeclampsia, eclampsia, and gestational hypertension, United States, 1987–2004. *Am J Hypertens* 2008; 21:521–526.
- Breathett K, Muhlestein D, Foraker R, et al. Differences in preeclampsia rates between African American and Caucasian women: Trends from the National Hospital Discharge Survey. *J Womens Health* 2014;23:886–893.
- Ananth CV, Keyes KM, Wapner RJ. Pre-eclampsia rates in the United States, 1980–2010: Age-period-cohort analysis. *BMJ* 2013;347:f6564.
- Hernandez-Diaz S, Toh S, Cnattingius S. Risk of preeclampsia in first and subsequent pregnancies: Prospective cohort study. *BMJ* 2009;338:b2255.
- Qiu C, Luthy DA, Zhang C, et al. A prospective study of maternal serum C-reactive protein concentrations and risk of preeclampsia. *Am J Hypertens* 2004;17:154–160.
- Rebello F, Schluskel MM, Vaz JS, et al. C-reactive protein and later preeclampsia: Systematic review and meta-analysis

- taking into account the weight status. *J Hypertens* 2013;31:16–26.
7. Lafayette RA, Druzin M, Sibley R, et al. Nature of glomerular dysfunction in pre-eclampsia. *Kidney Int* 1998;54:1240–1249.
 8. Moran P, Baylis PH, Lindheimer MD, et al. Glomerular ultrafiltration in normal and preeclamptic pregnancy. *J Am Soc Nephrol* 2003;14:648–652.
 9. Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: A meta-analysis. *Am J Kidney Dis* 2002;40:221–226.
 10. Countouris ME, Schwarz EB, Rossiter BC, et al. Effects of lactation on postpartum blood pressure among women with gestational hypertension and preeclampsia. *Am J Obstet Gynecol* 2016;215:241 e241–e248.
 11. Schwarz EB, Ray RM, Stuebe AM, et al. Duration of lactation and risk factors for maternal cardiovascular disease. *Obstet Gynecol* 2009;113:974–982.
 12. McClure CK, Catov JM, Ness RB, et al. Lactation and maternal subclinical cardiovascular disease among premenopausal women. *Am J Obstet Gynecol* 2012;207:46 e41–e48.
 13. Natland Fagerhaug T, Forsmo S, Jacobsen GW, et al. A prospective population-based cohort study of lactation and cardiovascular disease mortality: The HUNT study. *BMC Public Health* 2013;13:1070.
 14. Ridker PM, Rifai N, Rose L, et al. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 2002;347:1557–1565.
 15. Ridker PM, Danielson E, Fonseca FA, et al. Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: A prospective study of the JUPITER trial. *Lancet* 2009;373:1175–1182.
 16. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: Application to clinical and public health practice: A statement for health-care professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003;107:499–511.
 17. de Boer RA, Naylor M, deFilippi CR, et al. Association of cardiovascular biomarkers with incident heart failure with preserved and reduced ejection fraction. *JAMA Cardiol* 2018;3:215–224.
 18. Ridker PM, Buring JE, Rifai N, et al. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: The Reynolds Risk Score. *JAMA* 2007;297:611–619.
 19. Cook NR, Buring JE, Ridker PM. The effect of including C-reactive protein in cardiovascular risk prediction models for women. *Ann Intern Med* 2006;145:21–29.
 20. Fayh AP, Lopes AL, da Silva AM, et al. Effects of 5% weight loss through diet or diet plus exercise on cardiovascular parameters of obese: A randomized clinical trial. *Eur J Nutr* 2013;52:1443–1450.
 21. Kasapis C, Thompson PD. The effects of physical activity on serum C-reactive protein and inflammatory markers: A systematic review. *J Am Coll Cardiol* 2005;45:1563–1569.
 22. Kuzawa CW, Adair LS, Borja J, et al. C-reactive protein by pregnancy and lactational status among Filipino young adult women. *Am J Hum Biol* 2013;25:131–134.
 23. Luo J, Wang LP, Hu HF, et al. Cystatin C and cardiovascular or all-cause mortality risk in the general population: A meta-analysis. *Clin Chim Acta* 2015;450:39–45.
 24. van Holten TC, Waanders LF, de Groot PG, et al. Circulating biomarkers for predicting cardiovascular disease risk: a systematic review and comprehensive overview of meta-analyses. *PLoS One* 2013;8:e62080.
 25. Dent TH. Predicting the risk of coronary heart disease. II: The role of novel molecular biomarkers and genetics in estimating risk, and the future of risk prediction. *Atherosclerosis* 2010;213:352–362.
 26. Salgado JV, Franca AK, Cabral NA, et al. Cystatin C, kidney function, and cardiovascular risk factors in primary hypertension. *Rev Assoc Med Bras (1992)* 2013;59:21–27.
 27. Streven H, Wide-Svensson D, Grubb A. Serum cystatin C is a better marker for preeclampsia than serum creatinine or serum urate. *Scand J Clin Lab Invest* 2001;61:575–580.
 28. Yang X, Wang H, Wang Z, Dong M. Alteration and significance of serum cardiac troponin I and cystatin C in preeclampsia. *Clin Chim Acta* 2006;374:168–169.
 29. Kristensen K, Wide-Svensson D, Schmidt C, et al. Cystatin C, beta-2-microglobulin and beta-trace protein in preeclampsia. *Acta Obstet Gynecol Scand* 2007;86:921–926.
 30. Thilaganathan B, Ralph E, Papageorgiou AT, et al. Raised maternal serum cystatin C: An early pregnancy marker for preeclampsia. *Reprod Sci* 2009;16:788–793.
 31. ACOG Committee on Obstetric Practice. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. *Obstet Gynecol* 2002;99:159–167.
 32. American Academy of Pediatrics. Breastfeeding and the use of human milk. *Pediatrics* 2012;129:e827–e841.
 33. Pai JK, Pischon T, Ma J, et al. Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med* 2004;351:2599–2610.
 34. Cushman M, Arnold AM, Psaty BM, et al. C-reactive protein and the 10-year incidence of coronary heart disease in older men and women: The cardiovascular health study. *Circulation* 2005;112:25–31.
 35. Morgen CS, Bjork C, Andersen PK, et al. Socioeconomic position and the risk of preterm birth—A study within the Danish National Birth Cohort. *Int J Epidemiol* 2008;37:1109–1120.
 36. Thurston RC, Kubzansky LD, Kawachi I, et al. Is the association between socioeconomic position and coronary heart disease stronger in women than in men? *Am J Epidemiol* 2005;162:57–65.
 37. Schwarz EB, McClure CK, Tepper PG, et al. Lactation and maternal measures of subclinical cardiovascular disease. *Obstet Gynecol* 2010;115:41–48.
 38. Stuebe AM, Kleinman K, Gillman MW, et al. Duration of lactation and maternal metabolism at 3 years postpartum. *J Womens Health* 2010;19:941–950.
 39. Groer MW, Jevitt CM, Sahebzamani F, et al. Breastfeeding status and maternal cardiovascular variables across the postpartum. *J Womens Health* 2013;22:453–459.
 40. Visser M, Bouter LM, McQuillan GM, et al. Elevated C-reactive protein levels in overweight and obese adults. *JAMA* 1999;282:2131–2135.
 41. Christian LM, Porter K. Longitudinal changes in serum proinflammatory markers across pregnancy and postpartum: Effects of maternal body mass index. *Cytokine* 2014;70:134–140.
 42. Madsen C, Nafstad P, Eikvar L, et al. Association between tobacco smoke exposure and levels of C-reactive protein in the Oslo II Study. *Eur J Epidemiol* 2007;22:311.

43. Risch M, Purde MT, Baumann M, et al. High first-trimester maternal blood cystatin C levels despite normal serum creatinine predict pre-eclampsia in singleton pregnancies. *Scand J Clin Lab Invest* 2017;1–10.
44. Poole AT, Vincent KL, Olson GL, et al. Effect of lactation on maternal postpartum cardiac function and adiposity: A murine model. *Am J Obstet Gynecol* 2014;211:424 e421–e427.
45. Stuebe A. Associations among lactation, maternal carbohydrate metabolism, and cardiovascular health. *Clin Obstet Gynecol* 2015;58:827–839.
46. Gunderson EP, Hedderston MM, Chiang V, et al. Lactation intensity and postpartum maternal glucose tolerance and insulin resistance in women with recent GDM: The SWIFT cohort. *Diabetes Care* 2012;35:50–56.
47. ACOG. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol* 2013;122:1122–1131.

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