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Plasma Ferritin and Hepcidin Are Lower at 4 Months Postpartum among Women with Elevated C-Reactive Protein or α1-Acid Glycoprotein[1,2]

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Abstract

Background: Ferritin and hepcidin are markers of iron status that typically increase during inflammation or infection. The postpartum period is a physiologically unique life stage in which the relations between these proteins and other markers of inflammation have not been extensively studied.

Objective: We aimed to determine whether 2 markers of inflammation [high-sensitivity C-reactive protein (CRP) and α1-acid glycoprotein (AGP)] were associated with ferritin or hepcidin in postpartum women in California.

Methods: This is a secondary analysis of a randomized controlled iron-intervention trial. Plasma CRP, AGP, ferritin, and hepcidin were analyzed at 2 and 17 wk postpartum in 114 lactating women. We examined Pearson correlation coefficients between all biomarkers at both time points and differences in mean values of ferritin and hepcidin between those with and without elevated CRP and/or AGP.

Results: At 2 and 17 wk postpartum, 58% and 26% of women had CRP >5 mg/L and 78% and 29% had AGP >1 g/L, respectively. Neither CRP nor AGP was significantly correlated with ferritin ($r = 0.07$ and -0.06; $n = 114$ at 2 wk; -0.14 and -0.14; $n = 95$ at 17 wk) or hepcidin ($r = 0.18$ and -0.03 at 2 wk; -0.05 and -0.14 at 17 wk; $P > 0.05$ for all). At 2 wk, geometric mean plasma ferritin and hepcidin concentrations did not differ between women with and without elevated CRP or AGP ($P > 0.5$), but at 17 wk women with elevated CRP or AGP had lower mean (95% CI) ferritin and hepcidin than did women without either elevated CRP or AGP [ferritin: 30.3 ng/mL (23.4, 39.1 ng/mL) compared with 40.2 ng/mL (32.9, 49.2 ng/mL); $P < 0.01$; hepcidin: 44.3 ng/mL (32.3, 60.9 ng/mL) compared with 67.6 ng/mL (56.1, 81.5 ng/mL); $P = 0.02$].

Conclusion: Lower ferritin and hepcidin among women with elevated CRP or AGP at 17 wk postpartum suggests that these markers of iron status react differently to physiologic immune activation than to pathologic inflammatory states. *J Nutr* doi: 10.3945/jn.116.245803.

Keywords: iron, ferritin, hepcidin, inflammation, C-reactive protein, α1-acid glycoprotein, postpartum, breastfeeding, lactation

Introduction

Acute phase proteins (APPs)[6] are a class of proteins whose plasma concentrations increase or decrease in response to inflammation. Plasma ferritin and hepcidin are 2 iron-related APPs, which, in addition to increasing with increased iron status, have been shown to increase in an inflammatory state (1, 2). Plasma ferritin, a biomarker of iron storage, increases during infection as iron is sequestered, presumably to prevent pathogens from utilizing circulating iron for growth. Hepcidin is an iron-regulatory hormone that is stimulated by inflammation or by iron overload. It decreases iron absorption and circulating plasma iron by inhibiting the release of iron from gut enterocytes and reticuloendothelial cells (3).

Plasma C-reactive protein (CRP) and α1-acid glycoprotein (AGP) are APPs that are commonly used as indicators of inflammation or infection. CRP rapidly increases within hours of an inflammatory stimulus, reaching a peak 48 h after the initiation of hepatic synthesis. It has a relatively short half-life,

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TABLE 1 Characteristics of the study population at 2 and 17 wk postpartum[1]

Characteristics	2.0 ± 0.9 wk postpartum (n = 114)	17.3 ± 1.6 wk postpartum (n = 95)
Age, y	30.1 [28.6, 31.6]	—
BMI (n = 110), kg/m²	28.5 [27.1, 30.0]	27.7 [26.0, 29.5]
Education, y	16.0 [15.4, 16.7]	—
Children, n	1.8 [1.5, 2.0]	—
Cesarean delivery, %	21.9	—
Married or living as married, %	73.7	—
MediCal,[2] % yes	33.3	—
WIC,[3] % yes	34.2	—
Current smoker, % yes	7.0	—
Race/ethnicity, %		
African American	9.3	—
Asian American	12.7	—
White	61.9	—
Latino	15.3	—
Native American	0.9	—
Plasma ferritin, ng/mL	43.6 (35.2, 54.1)	38.8 (30.5, 49.4)
Hepcidin, ng/mL	36.9 (28.6, 47.6)	56.6 (43.4, 73.8)
Hemoglobin, g/L	134.5 [132.5, 136.5]	134.2 [132.6, 135.7]
Transferrin saturation, %	20.6 (17.7, 23.4)	22.9 (19.9, 25.9)
Plasma CRP, mg/L	6.8 (5.0, 9.3)	2.1 (1.4, 3.2)
Plasma AGP, g/L	1.23 (1.15, 1.31)	0.95 (0.91, 0.99)

[1] Values are geometric means (95% CIs), arithmetic means [95% CIs], or percentages. AGP, α1-acid glycoprotein; CRP, C-reactive protein; WIC, Special Supplemental Nutrition Program for Women, Infants, and Children.
[2] California Medical Insurance (Medicaid) Program that provides health insurance for people with low income.
[3] A federal assistance program for nutrition for low-income pregnant and breastfeeding women and infants and children <5 y of age.

so when the stimulus for production is eliminated, the circulating concentration decreases rapidly (4). AGP is an APP that increases more slowly: it increases ~24 h after the onset of infection and continues to be detectable weeks after the infection (5). Multiple studies in various populations have shown positive associations between CRP and AGP and both ferritin and hepcidin (6, 7). A meta-analysis of 32 studies confirmed that ferritin is elevated when CRP and/or AGP are elevated, and the authors suggested the use of correction factors to adjust ferritin values for inflammation when using ferritin as an indicator of iron status (2). Although infants, children, and women and men were represented in the 32 studies in the analysis, only 2 of the studies (274 of 8796 participants) included postpartum women. One of those 2 studies included HIV-positive women (8), although Thurnham et al. (2) considered HIV-positive (n = 69) and HIV-negative (n = 68) women separately in their analysis. The other study controlled for AGP in ferritin analysis without mentioning associations between ferritin and AGP and did not mention CRP in the article (9).

The postpartum period is a time of heightened inflammatory and immune responses, independent of overt infection. Groer et al. (10) showed that women at 4–6 wk postpartum had higher serum inflammatory markers and cytokines than did nonpregnant, nonpostpartum women. These differences are presumably related to the need to repair tissue damage caused during childbirth, protect the uterus from infection during involution, and boost immunity to enhance the mother's ability to protect and nurture her newborn (10). It is not clear whether this immune-activated state in postpartum women influences ferritin

and hepcidin concentrations, because it has been shown to occur during infection-induced inflammation in other populations. The objectives of this article were to examine associations between markers of inflammation (CRP and AGP) and markers of iron status, including ferritin and hepcidin, in postpartum, lactating women and to evaluate the need for correction factors to adjust ferritin on the basis of CRP and AGP during this unique phase of the life cycle.

Methods

The data presented in this article are from a randomized, placebo-controlled iron-intervention trial in postpartum women at the University of California, Davis, Medical Center (UCDMC) in Sacramento, California (11). Women included in the study were ≥18 y of age, planned to return to the UCDMC for future health care, consumed iron-containing prenatal vitamin-mineral supplements (PNVs) for ≥3 mo and 4 d/wk during pregnancy, and planned to breastfeed for ≥3 mo. Women whose hemoglobin concentration was <110 g/L were excluded from the study. All of the women gave written consent to participate in the study, which was approved by the Human Subjects Review Committee at the University of California, Davis.

Women enrolled in the study were randomly assigned in blocks of 12 to receive a daily oral dose of 1 of 3 regimens for a duration of 3 mo: 1) PNVs without iron (purchased commercially from GNC) and 27 mg Fe as iron sulfate (purchased commercially from Rite Aid) consumed with meals, 2) PNVs without iron and 27 mg Fe consumed between meals, or 3) PNVs without iron and a placebo capsule consumed between meals. Women in the iron-with-meals group were instructed to consume the capsules with dinner, whereas women in the iron-between-meals and placebo groups were instructed to consume the capsules at bedtime ≥2 h after consuming any food. The differences between intervention groups in mean hemoglobin, iron status, and inflammation are presented elsewhere (11).

Blood samples were collected at the baseline study visit and after 3 mo of the intervention. Participants were given a list of foods high in iron and were asked not to consume those foods on the day of the blood draws and not to consume any food or drink for 1 h before the scheduled time of the study visit. Those who failed to follow dietary instructions were asked to return for a later study visit. Venous blood was collected from the antecubital vein by licensed UCDMC phlebotomists into a potassium-EDTA tube (BD Vacutainer) and a trace mineral–free heparinized polypropylene syringe (Sarstedt Monovette, NH4-heparin; Sarstedt, Inc.). The potassium-EDTA blood tube was sent to UCDMC Pathology within 1 h for hemoglobin analysis. The heparinized tube was put on ice until centrifugation within 2 h at 1050 × g for 10 min at 4°C. Plasma was placed into aliquots in 2-mL cryovials and stored at −20°C until analysis for transferrin saturation (TfSat), ferritin, hepcidin, high-sensitivity CRP, and AGP.

TABLE 2 Prevalence of low or high circulating biomarkers at 2 and 17 wk among postpartum women in California[1]

	2.0 ± 0.9 wk postpartum (n = 114), %	17.3 ± 1.6 wk postpartum (n = 95), %
CRP >5 mg/L	58	26
AGP >1 g/L	78	29
CRP >5 mg/L or AGP >1 g/L	82	42
Hemoglobin <120 g/L	11	1
Ferritin <15 ng/mL	11	8
Hepcidin <8 ng/mL	8	4
TfSat <20%	53	40
MCV <80 fL/RBC	1	1
RDW >14.5%	17	11

[1] AGP, α1-acid glycoprotein; CRP, C-reactive protein; MCV, mean corpuscular volume; RDW, RBC distribution width; TfSat, transferrin saturation.

TABLE 3 Pearson correlations between circulating hemoglobin, markers of iron status, and inflammatory markers at 2 wk postpartum among women in California[1]

	Ferritin, ng/mL	TfSat, %	Hepcidin, ng/mL	Hemoglobin, g/L	CRP, mg/L
TfSat, %	0.46*				
Hepcidin, ng/mL	0.35*	0.23*			
Hemoglobin, g/L	0.42*	0.67*	0.23*		
CRP, mg/L	0.07	−0.45*	0.18	−0.32*	
AGP, g/L	−0.06	−0.32*	−0.03	−0.20*	0.66*

[1] n = 114. All data except for hemoglobin were log-transformed before analyses were performed. *P < 0.05. AGP, α1-acid glycoprotein; CRP, C-reactive protein; TfSat, transferrin saturation.

Hemoglobin was measured as part of a complete blood count by UCDMC Pathology (UniCel DxC 800; Beckman Coulter). TfSat was measured by an autoanalyzer at UCDMC Pathology (Beckman Coulter LH-785). Hemoglobin and TfSat were measured singly on instruments that were calibrated as needed, under a variety of circumstances (instrument maintenance, new reagent lot number, during troubleshooting of failed quality control). Quality control was performed multiple times on the day the samples were analyzed. CRP and AGP were measured turbidimetrically by an automated procedure (Cobas Integra Autoanalyzer) in which CRP was bound to mouse anti-CRP antibodies that were bound to latex, whereas AGP was bound to rabbit anti-AGP T antiserum specific for human AGP. Ferritin was measured by Coat-A-Count IRMA (Siemens). Hepcidin was measured by an enzyme immunoassay kit (Peninsula Laboratories). Ferritin, hepcidin, CRP, and AGP were analyzed in duplicate; and any samples with a high CV (CV >10%) were reanalyzed.

Data were analyzed by using SAS software versions 9.2 and 9.3 (SAS Institute). The sample size was based on the ability to detect a difference between intervention groups as described previously (11). The resulting sample size at enrollment (n = 114) was sufficient to detect a minimum correlation coefficient of 0.231 with power = 0.8. Data were tested for normality of distribution by using the Shapiro-Wilk test; data for ferritin, hepcidin, TfSat, CRP, and AGP were found to be not normally distributed and were thus log transformed before statistical analyses. Pearson correlation coefficients were used to determine correlations between variables at the initial and final study visits. The differences in geometric mean ferritin, hepcidin, and TfSat and mean hemoglobin between groups with and without either elevated CRP or AGP were examined by Student's t test. Each participant was then further categorized into 1 of 4 stages of inflammation on the basis of CRP and AGP values as described by Thurnham et al. (2). The categories were incubation (CRP >5 mg/L and AGP <1 g/L), early convalescence (CRP >5 mg/L and AGP >1 g/L), late convalescence (CRP <5 mg/L and AGP >1 g/L), and the reference category (CRP <5 mg/L and AGP <1 g/L). The geometric mean ferritin, hepcidin, and TfSat and mean hemoglobin values for each category were determined and compared with the values in the other categories by using ANOVA. The interaction between inflammation and mode of delivery (vaginal compared with cesarean delivery) was examined in the analyses of differences in means of outcome variables between those with and without elevated markers of inflammation. The differences between initial and final laboratory values were compared by using a paired t test.

Results

At the initial visit (mean ± SD 2.0 ± 0.9 wk postpartum), samples were collected from 114 women. At the follow-up visit (mean ± SD 17.3 ± 1.6 wk postpartum), samples were collected from 95 women (17% attrition). The average blood draw times were 1150 and 1157 for the 2- and 17-wk visits, respectively.

Characteristics of the study population at 2 wk postpartum are described in **Table 1**. Proportions of women with elevated CRP and/or AGP and RBC distribution width, as well as proportions of women with low hemoglobin, ferritin, hepcidin, TfSat, and mean corpuscular volume, are shown in **Table 2**. Both median CRP and AGP were significantly lower at 17 wk [2.3 mg/L (IQR: 0.9–5.3 mg/L) and 0.9 g/L (0.7–1.1 g/L), respectively] compared with 2 wk [7.7 mg/L (2.9–15.4 mg/L) and 1.2 g/L (1.0–1.5 g/L), respectively] (P < 0.01 for both).

At 2 wk postpartum, CRP and AGP were significantly correlated (P < 0.01; **Table 3**). There were significant positive correlations between ferritin and TfSat (P < 0.001), hepcidin (P < 0.01), and hemoglobin (P < 0.01), whereas no significant correlations were found between ferritin and either CRP (P = 0.48) or AGP (P = 0.51). In addition to the positive correlation between hepcidin and ferritin, there were positive associations between hepcidin and TfSat (P = 0.02) and hemoglobin (P = 0.01) at 2 wk. There was a trend toward a positive association between hepcidin and CRP at 2 wk (P = 0.06), but no correlation between hepcidin and AGP (P = 0.73). CRP and AGP were both negatively correlated with TfSat (P < 0.01 and P < 0.01, respectively) and hemoglobin (P < 0.01 and P = 0.03, respectively) at the initial study visit.

At 17 wk postpartum, CRP and AGP remained significantly correlated (P < 0.01; **Table 4**), but the negative correlation between both markers and hemoglobin seen at 2 wk no longer existed (P = 0.99 for CRP compared with hemoglobin; P = 0.98 for AGP compared with hemoglobin), nor did the positive correlation between ferritin and hemoglobin (P = 0.50). The correlation coefficients between ferritin and both CRP and AGP were negative, although not significant (P = 0.18 for both). There were no significant correlations between hepcidin and either CRP (P = 0.64) or AGP (P = 0.17) at the follow-up visit.

The geometric mean ferritin, hepcidin, and TfSat and mean hemoglobin values for the reference group and groups with elevated CRP and/or AGP are shown in **Table 5**. There were no interactions between inflammation and mode of delivery with regard to differences in the means of outcome variables between those with and without elevated markers of inflammation. There was no difference in geometric mean ferritin at 2.0 wk postpartum between the reference group and the group with either elevated CRP or AGP. At 17.3 wk postpartum, the group with either elevated CRP or AGP had significantly lower geometric mean ferritin than did the reference group. After separating those with elevated CRP or AGP into stage of inflammation, there were no significant differences in geometric mean ferritin between categories at either 2.0 or 17.3 wk postpartum. There was no difference in geometric mean hepcidin at 2.0 wk

TABLE 4 Pearson correlation between circulating hemoglobin, markers of iron status, and inflammatory markers at 17 wk postpartum among women in California[1]

	Ferritin, ng/mL	TfSat, %	Hepcidin, ng/mL	Hemoglobin, g/L	CRP, mg/L
TfSat, %	0.39*				
Hepcidin, ng/mL	0.32*	0.07			
Hemoglobin, g/L	0.07	0.05	0.04		
CRP, mg/L	−0.14	−0.43*	−0.05	0.001	
AGP, g/L	−0.14	−0.34*	−0.14	−0.01	0.57*

[1] n = 95. All data except for hemoglobin were log-transformed before analyses were performed. *P < 0.05. AGP, α1-acid glycoprotein; CRP, C-reactive protein; TfSat, transferrin saturation.

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TABLE 5 Circulating ferritin, hepcidin, hemoglobin concentrations, and TfSat at 2.0 and 17.3 wk postpartum among women in California categorized by inflammation stage with cutoffs for CRP at 5.0 mg/L and AGP at 1.0 g/L[1]

Study visit and outcome	Reference (neither CRP nor AGP elevated)		Either CRP or AGP elevated			Incubation (elevated CRP but not AGP)		Early convalescence (both CRP and AGP elevated)		Late convalescence (AGP elevated, but not CRP)		
	Mean (95% CI)	n	Mean (95% CI)	n	P[2]	Mean (95% CI)	n	Mean (95% CI)	n	Mean (95% CI)	n	P[3]
2.0 ± 0.9 wk postpartum (n = 114)												
Ferritin, ng/mL	43.9 (33.2, 58.0)	20	43.6 (37.0, 51.3)	94	0.97	75.3 (41.2, 137.7)	5	42.2 (34.4, 51.8)	61	42.3 (31.4, 51.0)	28	0.46
Hepcidin, ng/mL	32.9 (22.7, 47.6)	20	37.8 (31.3, 46.7)	94	0.54	37.7 (19.6, 72.7)	5	41.6 (32.7, 53.0)	61	30.7 (22.0, 42.8)	28	0.48
Hemoglobin, g/L	138.8 [135.5, 142.1]	20	133.6 [131.3, 135.9]	94	0.049	131.2 [120.8, 141.6]	5	132.3 [129.3, 135.4]	61	136.8 [133.2, 140.4]	28	0.055
TfSat, %	25.7 (20.9, 30.5)[a]	20	19.5 (17.4, 21.5)	94	0.01	17.6 (6.9, 28.2)[a,b]	5	17.2 (14.9, 19.5)[b]	61	24.9 (20.9, 28.9)[a]	28	<0.01
17.3 ± 1.6 wk postpartum (n = 95)												
Ferritin, ng/mL	40.2 (32.9, 49.2)	55	30.3 (23.4, 39.1)	40	<0.01	25.0 (16.5, 37.9)	12	38.7 (23.9, 62.7)	13	48.6 (32.1, 73.7)	15	0.17
Hepcidin, ng/mL	67.6 [56.1, 81.5][a]	55	44.3 (32.3, 60.9)	40	0.02	57.9 (33.3, 100.8)[a,b]	12	59.8 (33.3, 107.4)[a,b]	13	27.6 (17.7, 43.2)[b]	15	0.005
Hemoglobin, g/L	134.3 [132.3, 136.5]	54	134.0 [131.7, 136.3]	40	0.79	133.3 [128.7, 138.0]	12	131.5 [127.9, 135.2]	13	136.6 [132.4, 140.8]	15	0.34
TfSat, %	26.6 [24.1, 29.2][a]	54	18.1 (15.5, 20.8)	40	<0.01	14.1 (11.9, 16.3)[b]	12	18.9 (12.8, 24.9)[b]	13	20.7 (15.9, 25.6)[a,b]	15	<0.01

[1] Values are geometric means (95% CIs) or arithmetic means [95% CIs]. Means without a common superscript letter differ (P < 0.05). AGP, α1-acid glycoprotein; CRP, C-reactive protein; TfSat, transferrin saturation.
[2] Difference between the reference group and the group that had either elevated CRP or AGP as analyzed by Student's t test.
[3] Difference between the reference group and 3 stages of inflammation as analyzed by ANOVA.

postpartum between the reference group and the group with either elevated CRP or AGP. At 17.3 wk postpartum, the group with either elevated CRP or AGP had significantly lower geometric mean hepcidin than did the reference group. After separating those with elevated CRP or AGP into stage of inflammation, the geometric mean hepcidin of the late-convalescence group was significantly lower than the mean for the reference group (P < 0.01 for the overall model; P < 0.01 for late convalescence compared with reference) and tended to be lower than the mean for the early-convalescence group (P = 0.07). At 2 wk postpartum, mean hemoglobin was greater among women without elevated CRP or AGP than in those who had elevated CRP or AGP, but the differences were not significant when comparing the 4 categories of stages of inflammation. At 17 wk postpartum, there were no differences in hemoglobin between those with and without elevated CRP or AGP. At 2 wk postpartum, TfSat was lower among women with elevated CRP or AGP than in those without elevated CRP or AGP. Comparing the 4 categories of stages of inflammation, those with both elevated CRP and AGP had lower TfSat than those in the reference category and those with only elevated AGP (P < 0.01 for both). At 17 wk postpartum, those with elevated CRP or AGP continued to have lower TfSat than those without elevated CRP or AGP, and those with elevated CRP (with or without elevated AGP) had lower TfSat than those without elevated CRP or AGP (P < 0.01 for both).

Discussion

We found a high prevalence of elevated CRP and AGP in women at 2 and 17 wk postpartum, yet there were no significant correlations between either marker of inflammation and either ferritin or hepcidin at either the initial or final study visits. Geometric mean ferritin, hepcidin, and TfSat concentrations among women with either elevated CRP or AGP were lower than among women without either marker being elevated at 17 wk postpartum. These findings for ferritin and hepcidin are somewhat surprising, because studies in individuals in other life stages have shown both ferritin and hepcidin concentrations to be positively associated with markers of inflammation (2, 7, 12–17). Our results suggest that, among healthy postpartum women, ferritin and hepcidin are not positively associated with CRP and AGP as they are in other life stages.

Pregnancy and the postpartum period are unique immunologic states. During pregnancy, there is a shift from a predominance of T-helper 1 (cell-mediated) cytokines to a greater prevalence of T-helper 2 (humoral immunity) cytokines (18), which is believed to occur to protect the fetus (19). CRP, which is involved in the humoral response (20), has been shown to be higher in healthy pregnant women than in nonpregnant women (21). In the postpartum period, the immune system is activated, even in the absence of overt infection or inflammatory disease. The process of childbirth itself is associated with a significantly enhanced immune response in the first few days postpartum, even in the absence of a traumatic delivery (22, 23). Although the markers of inflammation decrease soon thereafter, the immune system remains activated throughout the first year postpartum. Proinflammatory cytokines, immune cells, and APPs have been shown to be elevated for up to 1 y postpartum (10, 24–27).

Studies that examined associations between inflammation and ferritin throughout the postpartum period are limited. A recent study conducted in the highlands of Bolivia showed that adjusting for inflammation resulted in lower mean ferritin

concentrations (and an increased prevalence of iron deficiency) among women at 1 mo and between 6 and 8 mo postpartum (28). A separate study in Zaire showed higher mean ferritin values among postpartum women with either moderate or severe inflammation than in women without inflammation (29). Both of these studies were conducted in areas with low socioeconomic resources where the prevalence of clinical or subclinical infection may have been higher than in our study population, perhaps causing ferritin to respond as it typically does during infection. Two additional studies in healthy postpartum women showed positive associations between CRP and ferritin (22, 23). It is important to note, however, that both of those studies were conducted within 48 h postpartum when CRP was remarkably elevated (32.3 and 52.7 mg/L compared with 7.7 mg/L at 2 wk in the current study). With such a heightened inflammatory response, ferritin increased as it does in times of overt infection or trauma (30). A meta-analysis by Thurnham et al. (2) showed that, in women from all life stages combined, ferritin was significantly higher in those who had elevated CRP and/or AGP, and the authors suggested the use of correction factors to adjust ferritin values among those with inflammation. However, that meta-analysis included only 2 studies in postpartum women. In the original reports for each of those 2 studies, descriptions of associations between elevated CRP and/or AGP and ferritin were not provided (8, 9). In a study not included in the meta-analysis, Zimbabwean women at 2–12 mo postpartum with elevated CRP (>10 mg/L) had lower mean ferritin than did women without elevated CRP (31), which is in line with our findings and contrary to the findings of the meta-analysis.

The only study we found that examined the associations between hepcidin and either CRP or AGP postpartum included women in the immediate postpartum period. van Santen et al. (32) found a trend ($r = 0.48$, $P = 0.08$) toward a positive correlation between CRP and hepcidin among 14 women at 24 h postpartum. In a separate study in 38 women at 3 d postpartum, Gyarmati et al. (33) found no correlation between hepcidin and IL-6, a cytokine that induces the synthesis of hepcidin during an inflammatory response, but neither CRP nor AGP was measured. Although there was no direct linear correlation between hepcidin and either CRP or AGP in our study, the geometric mean hepcidin among women in the late-convalescence category was significantly lower than among women in the reference category at 17 wk. This is the opposite of what would be expected during pathologic inflammation when hepcidin typically increases. We did find, however, significant positive correlations at both time points between hepcidin and ferritin, suggesting that hepcidin was influenced more by iron status (as it typically is in the absence of infection) than by inflammation.

The postpartum period is not the only time when inflammation occurs in the absence of overt infection. During pregnancy, CRP is typically elevated (21), but this may not always be "normal," because it is associated with adverse outcomes, such as gestational diabetes and preeclampsia (34) among mothers and fetal growth restriction and neonatal complications (35). Elevated CRP has been shown to be associated with ferritin concentrations among pregnant women in Guinea-Bissau (36), but was not associated with ferritin concentrations among pregnant Australian women (37). Given the lower socioeconomic status of the Guinea-Bissau population, it is possible that the association between CRP and ferritin was caused by acute infections or chronic illness. Postexercise is another period in which elevated inflammatory markers have been observed, even in the absence of infection. However, exercise causes muscle damage (38), so exercise-induced inflammation may be more akin to inflammation resulting from trauma than to a "normal" enhanced immune system. Advanced age is yet another life stage when inflammation occurs in the absence of overt infection (39). Both ferritin (40) and hepcidin (39, 41) have been shown to be disassociated with markers of inflammation among healthy elderly populations.

This study has limitations. The study population was a cohort of healthy women in California, all of whom had hemoglobin concentrations >110 g/L and consumed iron-containing PNVs during pregnancy. The results of this study may not be generalizable to other populations of lower socioeconomic status who may have higher rates of clinical or subclinical infection. In addition, although the geometric mean ferritin and hepcidin values were lower among the group with either elevated CRP or AGP, when comparing ferritin and hepcidin between the 4 categories of inflammation, the sample sizes in each of the categories may not have been large enough to detect differences. Similarly, the sample size was large enough to identify correlation coefficients of >0.23 as significant but not large enough to detect weaker correlations. We were further limited by the logistics of the blood collections. For the convenience of the new mothers, blood draws were scheduled one-half hour before the infant's scheduled well-child visit, which was, on average, just before noon. Because we could not reasonably ask lactating women to fast until late morning, blood draws were conducted in the nonfasted state. However, ferritin has not been shown to be acutely affected by a single high dose of oral iron (42), whereas hepcidin has been shown to be moderately elevated after iron consumption in women but not men (43). Nevertheless, to prevent postprandial increases in markers of iron status, we asked women to refrain from consuming iron-rich foods before the blood collection on study visit days and to not consume any food or drink for 1 h before the scheduled study visit. Any woman who reported that she did not follow these dietary instructions did not submit a blood sample and was asked to return at a later date for blood collection.

In conclusion, these results suggest that the activation of the immune system among healthy postpartum women is physiologically "normal" and does not cause an increase in hepcidin or ferritin as it does when pathologic inflammation is present. Further research is needed to examine the associations between various markers of inflammation and both ferritin and hepcidin in a variety of populations in both developed and developing countries.

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