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1Effect of iron supplementation during lactation on maternal iron status and oxidative stress: a randomized controlled trial.

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Abstract

We examined the effect of iron-containing prenatal vitamin-mineral supplements (PNV) taken postpartum on biomarkers of iron status and oxidative stress. Lactating women (n=114) were randomly assigned to consume daily one iron-free PNV plus either 27 mg of iron or placebo for approximately 3.5 mo. The placebo group took the tablets between meals, while those given iron took the tablets either with (Fe-W) or between meals (Fe-B). Blood and urine samples were collected before and after the supplementation period to analyze hemoglobin (Hb), ferritin, hepcidin, transferrin saturation (TfSat), total plasma iron, and biomarkers of oxidative stress (isoprostane and 8-hydroxy-2-deoxyguanosine (8-OHdG)) and inflammation (C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP)).

There was a trend toward a greater change in Hb among women in the Fe-B group compared to placebo (+2.5 vs. -3.7 g/L, respectively, P=0.063). When the iron groups were combined, there was a greater change in Hb (+1.4 g/L) compared to placebo (P=0.010). There were trends toward greater changes in TfSat (P=0.087) and total plasma iron (P=0.065) in the iron groups compared to placebo, yet no significant differences between the 3 groups in change in hepcidin (P=0.291), isoprostane (P=0.319) or 8-OHdG.
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Iron consumption during lactation moderately increased iron status, particularly among women without elevated CRP, and increased Hb, but did not significantly increase oxidative stress.

Keywords: Iron, oxidative stress, inflammation, postpartum, breastfeeding, lactation
Introduction

Both the Centers for Disease Control and Prevention and World Health Organization recommend iron supplementation during pregnancy to prevent iron-deficiency anemia in both mother and child (Prevention 1998, Moran et al. 2007). The Recommended Dietary Allowance (RDA) for iron is 27 mg/d during pregnancy and 18 mg/d for non-pregnant, non-lactating women (Trumbo et al. 2001). By contrast, the RDA for iron in lactation is only 9 mg/d because of the expectation that there will be no menstrual losses during the first 6 mo postpartum, and the iron accumulated during prenatal formation of maternal red blood cells can be recycled and used by the mother postpartum (Milman 2011). As a result, universal iron supplementation is generally not necessary for healthy lactating women. Despite this, many women are advised to continue taking prenatal vitamin-mineral supplements (usually containing at least 30 mg iron daily) for the duration of lactation. Data from the third National Health and Nutrition Examination Survey (NHANES) showed that, on average, lactating women took about 30 mg iron from supplements in addition to their daily dietary iron intake (~ 16 mg/d) (Heimbach 2001) – the combination of which exceeds the Tolerable Upper Intake Level (UL) of 45 mg/d specified for pregnant and lactating women.

For women who have become iron deficient during pregnancy or experienced substantial blood loss during childbirth, continuation of iron supplements postpartum may be beneficial. However, for those who have adequate iron reserves after childbirth, iron supplements could pose some risk. Iron is able to exist in multiple valency states, which allows it to donate and accept electrons. Via the Fenton reaction, iron can donate an electron to hydrogen peroxide, forming a hydroxyl radical (Gutteridge 1986, Gutteridge et al. 1985, Burkitt and Mason 1991). The hydroxyl radical can initiate oxidation of various cellular components, including lipids and DNA, which can lead to cellular and tissue damage (Dargel 1992, Cooke et al. 2003). Reactive oxygen...
species have been linked to hepatotoxicity (Videla et al. 2003, Uchiyama et al. 2008, Jaeschke et al. 2002), some types of cancer (Poulsen et al. 1998, Valavanidis et al. 2009), metabolic syndrome (Holvoet et al. 2008, Leiva et al. 2013), subsequent atherosclerosis (Hulsmans and Holvoet 2010, Holvoet 2004, Holvoet et al. 2004), and even periodontitis (Itabe 2012), although it is often difficult to discern whether reactive oxygen species are a cause or effect of such pathologies.

Risks of excess iron consumption may be exacerbated by consuming iron-containing supplements between meals as is commonly advised to increase absorption. Plasma iron concentration peaks at a higher level when consumed between-meals compared to with-food (Kamp et al. 2003), which may increase the amount of unbound iron in circulation that is able to participate in oxidation reactions.

The aim of this trial was to investigate the effects of daily consumption of prenatal vitamins with or without iron during lactation on hemoglobin (Hb), iron status, and two markers of oxidative stress. In addition to addressing the hypothesis that women supplemented with iron will have higher concentrations of Hb, iron status, and oxidative stress compared to those given placebo, we explored the hypothesis that those who consume iron between meals will have higher markers of oxidative stress than those who consume iron with meals.
Methods

This study was a randomized, partially blind intervention trial of lactating women in Sacramento, California. Women less than 2 wk postpartum were recruited from the labor and delivery ward and the pediatric outpatient clinics at UC Davis Medical Center (UCDMC) from October 2008 until April 2010. Women included in the study were at least 18 years of age, planned to return to UCDMC for future health care, consumed iron-containing prenatal vitamin mineral supplements (PNV) for at least 3 mo and 4 d/wk during pregnancy, and planned to breastfeed for at least 3 mo. Women whose Hb concentration was less than 110 g/L were excluded from the study.

Women were randomly assigned in blocks of 12 to 1 of 3 intervention groups by selecting a sealed envelope produced by study coordinators that contained within it the intervention group. Blocking was purely random and not based on any participant information, only to ensure equal sample sizes across the intervention groups over time. Women were assigned to a daily oral dose of 1 of 3 regimens for approximately 3.5 months: 1) PNV without iron (procured from GNC, Pittsburgh, PA) and 27 mg of iron as iron sulfate (procured from Rite Aid, Harrisburg, PA) consumed with meals (Fe-W); 2) PNV without iron and 27 mg of iron as iron sulfate consumed between meals (Fe-B); 3) PNV without iron and placebo (Placebo) consumed between meals.

The PNV without iron and iron supplements were produced commercially and are safe for human consumption. The iron tablets were ground down and repacked by Investigational Drug Services (IDS) at UCDMC into capsules that were identical in size and appearance to the placebo capsules (methylcellulose powder) that were also produced by IDS. Women in the Fe-W group were instructed to consume the capsules with dinner, while women in the Fe-B and Placebo groups were instructed to consume the capsules at bedtime at least 2 h after consuming.
any food. All participants agreed to not consume other vitamin or mineral supplements while participating in the trial. The baseline study visit was scheduled at the time of the infant’s 2 week well-child physician visit, and the follow up visit was scheduled at the infant’s 4 month well-child visit. All women gave written consent to participate in the study. The study was approved by the Human Subjects Review Committee at UC Davis.

Study coordinators were considered “partially blind”, as they did not know group assignment for those in the “between-meals” groups (Fe-B and Placebo) until after the code was broken. Research assistants were unaware of the lack of a “with-meals” placebo group, and thus were assumed to be completely blinded to group assignment. Participants were followed through a phone call conducted by study coordinators one week after enrollment in the study and monthly phone calls thereafter. Study coordinators inquired about compliance with supplement consumption, breastfeeding status, and general health during the preceding period.

Blood and urine samples were collected at the baseline study visit and after approximately 3.5 mo of intervention. Urine was collected at home by the mother on the morning of her scheduled study visit. Women were instructed to collect a complete bladder evacuation of the first urine of the morning into an 800 mL urine collection receptacle and then transfer 50 mL into a sterile urine collection cup, which was stored in the refrigerator until leaving for her study appointment. Venous blood was collected from the antecubital vein by licensed UCDMC phlebotomists into a potassium-EDTA tube (BD Vacutainer, Franklin Lakes, NJ) and a trace mineral-free polypropylene syringe (Sarstedt Monovette, NH4-heparin, Sarstedt Inc., Newton, NC). Before blood draws on study visit days, participants were instructed to refrain from consuming foods high in iron (based on a pre-specified list of such foods) and to not consume any food or drink for an hour before each study visit. Those who failed to follow urine collection and dietary
instructions were asked to continue consuming their assigned intervention and return for a later study visit.

Urine samples were put on ice upon arrival at the study site and aliquotted within 2 h into cryovials containing 0.005% BHT in methanol and stored at -80°C until analysis. Urine samples were used to analyze 8-isoprostane, 8-hydroxy-2-deoxyguanosine (8-OHdG), and creatinine. Isoprostanes are products of peroxidation of polyunsaturated fatty acids and are considered the best marker of lipid peroxidation (Halliwell and Whiteman 2004, Vincent et al. 2007). 8-OHdG is the most commonly analyzed marker of oxidized DNA damage due to its relative abundance (Cooke et al. 2002) and relative ease of detection (Chiou et al. 2003). The potassium-EDTA blood tube was sent to UCDMC Pathology within 1 h for Hb analysis. The heparinized tube was put on ice until centrifugation within 2 h at 2,500 rpm for 10 min. Plasma was aliquotted into cryovials and stored at -20°C or -80°C until analysis. Plasma samples used to analyze total antioxidant status (TAS) were stored at -80°C until analysis. TAS is the measurement of the quantity of a given free radical scavenged by a test solution, and therefore measures the antioxidant activity of the sum of all antioxidants in a sample, rather than measuring one or a limited number of specific antioxidant components (Ghiselli et al. 2000). Plasma samples stored at -20°C were used to analyze plasma iron, transferrin saturation (TfSat), ferritin, hepcidin, C-reactive protein (CRP), alpha-1-acid glycoprotein (AGP), aspartate transaminase (AST), and alanine transaminase (ALT). TfSat and ferritin are commonly used as markers of iron status, whereas hepcidin is a more recently discovered protein that functions as a key regulator of iron homeostasis (Collins et al. 2008). Hepcidin has been shown to increase with iron loading and decrease with iron deficiency (Darshan and Anderson 2009, Ganz 2011), making it an additional marker of whole-body iron status. AST and ALT are transaminases that increase with liver
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Damage, and have been shown in animal models to be associated with iron-induced oxidative stress (Asare et al. 2009).

Hb was measured as part of a complete blood count by UCDMC Pathology (Beckman Coulter UniCel DxC 800, Brea, CA). Total plasma iron, TfSat, AST, ALT, and urinary creatinine were measured by an Autoanalyzer at UCDMC Pathology (Beckman Coulter LH-785). CRP, AGP, and TAS were measured by an automated procedure (Cobas Integra Autoanalyzer, Indianapolis, IN). Ferritin was measured by Coat-A-Count IRMA (Siemens, Los Angeles, CA). Hepcidin was measured by EIA kit (Peninsula Laboratories, San Carlos, CA). Isoprostane and 8-OHdG were measured by EIA kits (Cayman Chemical, Ann Arbor, MI). Isoprostane, 8-OHdG, ferritin, hepcidin, CRP, and AGP were analyzed in duplicate and any sample with a high coefficient of variation (CV > 10%), both baseline and final samples were reanalyzed in the same batch. Both isoprostane and 8-OHdG had high between-plate (29.1% and 25.7%, respectively) and within-plate (11.7% and 10.7%, respectively) coefficients of variation, with multiple attempts required for several samples in the isoprostane analysis to fall within the standard curve. To account for the large between-plate variation, baseline and final samples for each participant were analyzed on the same plate, and for any sample that had a CV > 10%, both baseline and final samples were reanalyzed on the same plate.

Iron intake was assessed at the baseline and final study visits by asking the frequency of consumption of iron-rich foods. The questionnaire was based on a validated food frequency questionnaire, from which the same response options and iron-rich food categories were used (Block et al. 1990).

Statistical methods:
We determined a sample size of 32 per group for each of the 3 groups, based on the ability to detect a difference of ≥ 7.2 g/L in Hb concentration (alpha = 0.05; 80% power; assuming a standard deviation of 10 g/L), similar to the difference found between iron and placebo groups of pregnant women (Hoa et al. 2005). The number enrolled was increased by 10% to allow for attrition, bringing the needed sample size to 35 per group, or a total of 105 enrolled. Since randomization was done in blocks of 12, the total enrollment goal was increased to 108, or 36 per group to attain a multiple of 12.

Data were tested for normality of distribution using the Shapiro-Wilk test. Ferritin, hepcidin, TfSat, plasma iron, CRP, AGP, AST, ALT, TAS, isoprostane, and 8-OHdG were found to be non-normally distributed and were log transformed before statistical analyses. Treatment effects were examined by using intention-to-treat analysis. The change from baseline to final study visit for each continuous outcome variable was analyzed by analysis of covariance (ANCOVA), with the main effect being treatment group and controlling for baseline status of each variable and chosen covariates. In randomized studies the ANCOVA method is generally recommended for being unaffected by correlation between baseline and endline measurements, being consistent regardless of chance baseline measurement group imbalances, and generally having more power to detect group differences than repeated measures approaches (Vickers and Altman 2001; Van Breukelen 2006). For the ANCOVA method with the baseline measurement as a covariate, modelling the final measurement and the change in measurements is equivalent. The covariates included in the ANCOVA models have been shown in prior work to influence the outcome and upon bivariate analysis were significantly associated (p<0.10) with the specific outcome of interest in that model. Differences in outcome variables were examined between each of the three groups, as well as between the placebo group and the combined iron groups.
To examine effect modification, two-way interactions between group assignment and both ferritin and hepcidin at baseline were separately included in the ANCOVA model for change in isoprostane and 8-OHdG. Similarly, two-way interactions between group assignment and baseline CRP, AGP, and BMI were separately included in the ANCOVA model for change in ferritin, TfSat, total plasma iron, and hepcidin.

For all analyses, when the overall model was significant (\(P<0.05\)), groups were compared by using Tukey post hoc multicomparison test for ANOVA or with Bonferroni correction for ANCOVA. Logistic regression was used to determine the group-wise differences in proportion of participants that had values above or below chosen cut-offs at the final study visit after controlling for the baseline category (low or high) and covariates chosen as described above. Statistical analysis was carried out with the SAS 9.2 and 9.3 (SAS Institute Inc., Cary, NC) software packages.

**Results**

In total, 514 women were approached for recruitment into the study (Figure 1). Of the 174 women who were interested in participating at the time of recruitment, 57 could not be reached subsequently for the enrollment visit; thus Hb was assessed in 117 participants. Of those, 3 had Hb levels less than 110 g/L and were referred for care and excluded from the study.

Baseline demographic characteristics of all women who completed follow up in each of the intervention groups are displayed in Table 1. More women were lost to follow-up in the iron groups than in the placebo group (Figure 1; \(P=0.048\) for the overall 3-group model; \(P=0.014\) for Fe-B vs. Placebo; \(P=0.047\) for Fe-W vs. Placebo; \(P=0.017\) for the combined iron groups vs. Placebo), therefore, mean baseline demographic and laboratory values were compared between
women who completed follow up and those who were lost to follow up, and no significant differences were found (P>0.20 for all). Of those who completed the final study visit (Placebo: n=39; Fe-B: n=27; Fe-W: n=29), 2 women in the placebo group, and 3 in each of the Fe-B and Fe-W groups did not continue supplement consumption until the final study visit (P=0.640; Figure 1). Among women who continued supplementation until the final study visit, there were no significant differences among groups in the mean number of days women were enrolled in the study (Placebo: 107.4 d; Fe-B: 104.8; Fe-W: 109.9; P=0.166) or the mean number of reported days supplements were consumed (Placebo=98.3 d; Fe-B=96.9 d; Fe-W=99.4 d; P=0.763).

Among all women who completed the final study visit, there were no differences in the frequency of iron-rich food consumption according to the modified food frequency questionnaire (Placebo=27.5 response points; Fe-B=27.1 response points; Fe-W=27.2 response points; P=0.951), percentage of women who continued to breastfeed throughout the intervention period (Placebo=89.3%, Fe-B=87.2%, Fe-W=83.3%; P=0.794), or percentage of women who resumed menstruation by the final study visit (Placebo=44.4%, Fe-B=52.6%, Fe-W=46.7%; P=0.788).

There were no differences in the mean blood draw times between groups for either the baseline (11:50 AM; P=0.383) or final (11:57 AM; P=0.589) blood draws.

The mean (SD) changes from baseline to the final study visit in Hb, TfSat, total plasma iron, 8-OHdG, isoprostane, TAS, CRP, AGP, AST, and ALT are shown in Table 2. Hb increased in the Fe-B (+2.5 g/L) and Fe-W (+0.4 g/L) groups, and decreased in the placebo group (-3.7 g/L; adjusted P=0.034 for the overall model; Placebo vs. Fe-B, P=0.063; Placebo vs. Fe-W, P=0.114; adjusted P=0.010 for Placebo vs. the combined iron groups). There were marginally significant changes in ferritin between the baseline and final visits among the 3 intervention groups (P=0.091). Compared to the placebo group, the change in ferritin was marginally significantly
greater in the Fe-W group ($P=0.087$). There were trends toward a greater decline in ferritin in the placebo group and compared to the combined iron groups (-9.2 ng/mL vs. -3.3 ng/mL; adjusted $P=0.056$). There were no differences between groups in change in hepcidin, TfSat, total plasma iron, 8-OHdG, isoprostane, TAS, CRP, AGP, AST, or ALT.

In examining the differences between groups in change in ferritin, an interaction was found between treatment group and baseline CRP ($P=0.028$) when the iron groups were combined (2-group analysis) (Table 3). Among those with CRP ≤ 5 mg/L (n=38), ferritin increased significantly more in the combined iron groups compared to placebo ($P=0.001$), while there were no differences between groups in change in ferritin among those with elevated CRP (> 5 mg/L; n=57). There were no other significant interactions.

The percentages of women who had ferritin, hepcidin, or Hb below chosen cut-offs, or 8-OHdG, isoprostane, CRP or AGP above chosen cut-offs are shown in Table 4. At the final study visit, there was significant difference in the percentage of 8-OHdG greater than 1 SD above the mean among the 3 intervention groups (13% vs 11% vs 31%; adjusted $P=0.043$). There was a trend toward a greater percentage of women with higher 8OHdG in the Fe-W group compared to the Fe-B group (31% vs 11%; adjusted $P=0.062$ for Fe-W vs. Fe-B) and placebo group (31% vs 13%; $P=0.086$ for Fe-W vs. Placebo). There were no differences among groups in the proportion of women with elevated isoprostane. At the final study visit there were no significant differences among groups in the percentage with ferritin < 15 ng/mL, which is a cut-off value commonly used to define iron deficiency. When the combined iron groups were compared to placebo, there was a trend toward a greater proportion of women with ferritin < 15 ng/mL in the placebo group (13% vs. 5%; $P=0.070$). When a cut-off of 30 ng/mL was used, a cut-off shown to have higher sensitivity (without compromising specificity) for detecting iron deficiency (Mast et al. 1998),
the percentage of women in the placebo group with low ferritin tended to be greater than in the Fe-B group (46% vs. 29% vs. 21%; overall adjusted $P=0.019$; Fe-B vs. Placebo, adjusted $P=0.050$). When the iron groups were combined, a greater proportion of women in the placebo group had ferritin < 30 ng/mL compared to the combined iron group (46% vs. 25%; $P=0.006$). After controlling for the baseline category, there were no significant differences in the proportion of women who had hepcidin below 8 ng/mL ($P=0.997$), which is the cut-off commonly used to detect iron deficiency. Similarly, there were no significant differences among groups when using a cut-off of 18 ng/mL ($P=0.549$), a value shown to score higher than 8 ng/mL on the Youden Index used to determine an optimal cut-off value (Pasricha et al. 2011, Fluss et al. 2005). There were no differences among groups in the percentage of women with low Hb or high CRP or AGP.
Discussion

To our knowledge, this is the first study to report the effects of oral iron supplementation on iron status, oxidative stress, and markers of inflammation in postpartum women. The results indicate that daily iron supplementation for approximately 3.5 mo at doses typically found in prenatal vitamins increased Hb and resulted in a lower percentage of women with a ferritin level indicative of iron deficiency. However, only one woman in the placebo group had a Hb value less than 120 g/L at the end of the intervention period, which indicates that the lower iron status among women in the placebo group was generally not severe enough to cause iron deficiency anemia. We did not find significant differences in the change from baseline in markers of iron status or oxidative stress between the group taking iron between meals and the group taking iron with meals; however, the proportion of women with elevated 8-OHdG tended to be greater in the Fe-W group than either the Fe-B or placebo groups.

Our primary hypothesis was that iron supplementation of lactating women would increase oxidative stress, as the excess iron consumed would cause free-radical oxidation via the Fenton reaction. Previous reports on the association between iron status and both 8-OHdG and isoprostane in humans are mixed. Cross-sectional studies have shown positive associations between iron status and these markers of oxidative stress (Nakano et al. 2003, Tuomainen et al. 2007, Hori et al. 2010, Crist et al. 2009, Signorini et al. 2008). However, intervention studies that have investigated the effect of iron supplementation on 8-OHdG and isoprostane in humans have yielded mixed results – either no change in 8-OHdG or isoprostane after relatively high-dose iron supplementation (Orozco et al. 2012, Braekke et al. 2007), or a two-fold increase in 8-OHdG and isoprostane after consuming 120 mg of iron for 7 days (Schumann et al. 2005). It is
important to note that the supplementation period of each of these studies was only 7 days. With such a short intervention period, it is possible that the antioxidant capacities in these individuals were not overwhelmed and were able to quench iron-induced free radicals before 8-OHdG or isoprostane levels were affected. However, in our study, we also found no difference among groups in the mean values of markers of oxidative stress between iron and placebo groups after 3.5 mo of supplementation. It is likely that the antioxidants in the PNVs consumed by the women (ascorbic acid, α-tocopherol, β-carotene, selenium), elevated the levels of TAS and prevented oxidative stress in all 3 groups. In fact, the levels of TAS at the end of the supplementation period in this study were much higher than levels reported elsewhere in healthy postpartum women who did not consume vitamin-mineral supplements (Hung et al. 2010, Schulpis et al. 2007, Schulpis et al. 2008, Zarban et al. 2009). We did, however, see a trend toward higher 8-OHdG in the Fe-W group compared to both Fe-B and placebo groups, which is contrary to our hypothesis – that those in the Fe-B group would have higher oxidative stress than either of the other two groups. Consuming iron between meals has been shown to be associated with elevated plasma iron (Kamp et al. 2003), which we hypothesized would be associated with an increase in oxidative stress, as plasma iron has been shown to be positively associated with isoprostane (Crist et al. 2013), thio-barbituric-acid-reacting-substances (TBARS) (Viteri et al. 2012), and lipid peroxidation (King et al. 2008). Conversely, consuming iron with meals has been shown to cause oxidation of dietary cholesterol and lipids (Lorrain et al. 2012), which once absorbed, become incorporated into chylomicrons and low-density lipoproteins (Staprans et al. 2003), leading to increased risk of cardiovascular disease (Staprans et al. 2005). To our knowledge, ours is the first study to find evidence that suggests that consuming iron supplements with food rather than between meals may increase systemic oxidative stress.
We also examined the effect of iron supplementation on iron status. While the finding of increased iron status among those who consumed iron was expected, a surprising number of studies have reported no differences in iron status between iron and placebo groups among lactating women (Baykan et al. 2006, Powers et al. 1985, Correia-Santos et al. 2011, Stuetz et al. 2012, Mello-Neto et al. 2012). The lack of increase in iron status in these studies, and the lack of significant change in TfSat and plasma iron in the current study may be explained by the dynamic state of iron status in postpartum women. During pregnancy, the requirement for dietary iron increases as maternal and fetal tissue synthesis increase. Much of the additional iron required is used to increase the mother’s red cell mass. After giving birth, the red cells are broken down and the Hb iron contained within them is available for use or storage (Milman 2011).

Even without iron supplements postpartum, an increase in Hb and serum ferritin from delivery up to 8 wk postpartum in women who consumed iron during pregnancy has been described (Milman 2006). It is likely that the iron stored in the expanded red cell mass in women in the placebo groups in these studies was sufficient to prevent significant declines in iron status during the intervention period relative to the iron groups.

Previous studies that have compared changes in iron status after consuming supplemental iron with or between meals are limited and have not been conducted with lactating women (Domellof et al. 2008, Cook and Reddy 1995, Kamp et al. 2003, Dawson et al. 1998, Dawson et al. 2000). Authors of all but one of the studies reported either increased ferritin or iron absorption when iron was consumed between meals compared to with meals. It is important to note that none of the studies, including the present study, had a group size greater than 35. Future studies would benefit from a larger sample size to determine if there are in fact differences in iron status after a lengthy intervention of iron supplementation with or between meals.
There were significant interactions between treatment group and baseline CRP with regard to the change in ferritin. For those without elevated CRP at the baseline visit (40% of those enrolled), the combined iron groups had a greater change in mean ferritin than the placebo group. However, among those with high CRP at baseline (60% of those enrolled), there was no discernible effect of iron supplementation on ferritin, suggesting that the acute phase reaction interfered with an effect of iron supplementation on iron status.

Greater proportions of women in both of the iron groups were lost to follow up than in the placebo group. The reasons for this are not known. However, among those who discontinued supplementation yet remained in the study, the main reason for discontinuing supplementation was gastrointestinal upset. Health care providers recommend continuation of PNVs postpartum because of the high requirements for many nutrients during lactation. The vast majority of PNVs on the market contain iron. Assuming our results are generalizable, gastrointestinal upset caused by iron-containing PNVs may be inhibiting some women from consuming PNVs.

Although this study was a randomized, placebo-controlled study, it was not without limitations. First, while the sample size was large enough to detect differences in Hb, it likely was not large enough to detect modest differences in other outcomes and between the two groups differing in the timing of iron supplementation (with vs. between meals). Secondly, participants were given relatively complicated instructions for in-home collection of urine samples, which could have led to variability in collection technique, timing, and storage. We attempted to limit the variability by asking details about the collection, and if instructions were not followed, the participant was asked to recollect and submit a sample on a later day. Diurnal variations in urinary markers of oxidative stress have been reported (Helmersson and Basu 1999, Kanabrocki et al. 2002, Kanabrocki et al. 2006), although no significant differences were found between markers.
collected from 24-h urine collections and complete bladder collections of the first urine of the morning (Miwa et al. 2004, Basu 2008). Third, for convenience of the new mothers, blood draws were scheduled one half hour before the child’s doctor appointment. This meant that blood draws were non-fasting and varied as to the time of day samples were collected, although women were instructed to consume nothing but water for one hour before the blood draw, and to refrain from consuming iron-rich foods prior to the blood draw to prevent a postprandial spike in plasma iron. Those who consumed foods or beverages within the hour before the blood draw, or iron-rich foods on the day of the blood draw did not submit a blood sample and were asked to return at a later date for collection. Diurnal variation has been reported for plasma iron (Wiltink et al. 1973), hepcidin (Schaap et al. 2012), and TfSat (Wish 2006) but no diurnal variation has been shown in Hb or ferritin (Fleming et al. 2001). Moreover, there were no differences among groups in the average time of day of sample collection. Fourth, women who consumed iron between meals were instructed to do so at least 2 hours after dinner. It is possible that there was still food present in the stomach, particularly if the meal was a large one. This may have minimized the differences between the with- and between-meals groups. Fifth, the kits used for the isoprostane and 8-OHdG analyses had high between-plate (29.1% and 25.7%, respectively) and within plate (11.7% and 10.7%) coefficients of variation, with multiple attempts required for several samples in the isoprostane analysis to fall within the standard curve. To account for the large between-plate variation, baseline and final samples for each participant were analyzed on the same plate. Future analyses may benefit from using alternate methods of analyzing isoprostane and 8-OHdG.

In conclusion, among this population of postpartum women, iron supplementation led to a moderate increase in iron status, particularly among women without elevated CRP, yet no
difference in markers of oxidative stress between intervention groups. These data suggest that the current practice of recommending that mothers continue to consume iron-containing PNV while lactating likely outweigh the potential harms of increased oxidative stress. More research is needed to examine whether consuming iron between meals is safer than consuming iron with meals in order to prevent oxidation of dietary lipids and subsequent oxidation of endogenous lipoproteins. Future intervention studies should also consider inflammatory status when examining the effect of iron supplementation on iron status among postpartum women.

Additional research with larger sample sizes is needed to evaluate the effect of consuming iron supplements with or between meals on iron status, markers of oxidative stress and inflammation in postpartum women.

Key messages:

Breastfeeding women who consumed iron supplements from 2 to 17 weeks postpartum had a greater increase in Hb compared to women who consumed placebo, yet only one woman in the placebo group was mildly anemic at the end of the study (Hb < 120 g/L).

There were no differences in Hb or markers of iron status between women who consumed iron with vs. between meals.

Women who consumed iron with meals tended to have higher urinary 8-OHdG compared to women who consumed iron between meals or placebo.

The benefits of consuming iron-containing prenatal vitamin-mineral supplements while breastfeeding outweighed the potential harm of oxidative stress in this study population.
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Table 1 Baseline characteristics of the women by group for all women enrolled in the study separated into those who completed follow up and those who were lost to follow up.

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<th>Placebo (n=39)</th>
<th>Fe-B (n=28)</th>
<th>Fe-W (n=29)</th>
<th>Fe groups combined (n=57)</th>
<th>Completed follow-up (n=96)</th>
<th>Lost to follow-up (n=18)</th>
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<td>Age (y)</td>
<td>30.6 (5.2)²</td>
<td>29.4 (6.2)</td>
<td>31.0 (5.3)</td>
<td>30.2 (5.7)</td>
<td>30.3 (5.5)</td>
<td>28.7 (4.7)</td>
<td>0.256</td>
</tr>
<tr>
<td>Day postpartum</td>
<td>12.5 (5.3)</td>
<td>13.1 (6.0)</td>
<td>15.2 (8.1)</td>
<td>14.2 (7.2)</td>
<td>13.5 (6.5)</td>
<td>15.6 (6.2)</td>
<td>0.205</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.8 (5.7)</td>
<td>28.8 (4.7)</td>
<td>29.6 (4.8)</td>
<td>29.2 (4.8)</td>
<td>28.6 (5.2)</td>
<td>27.9 (5.1)</td>
<td>0.638</td>
</tr>
<tr>
<td>Total years of education</td>
<td>16.2 (2.7)</td>
<td>16.2 (2.1)</td>
<td>16.0 (1.6)</td>
<td>16.1 (1.8)</td>
<td>16.1 (2.2)</td>
<td>15.5 (2.7)</td>
<td>0.257</td>
</tr>
<tr>
<td>Number of children</td>
<td>1.8 (0.7)</td>
<td>1.9 (1.3)</td>
<td>1.7 (1.0)</td>
<td>1.8 (1.2)</td>
<td>1.8 (1.0)</td>
<td>1.7 (0.8)</td>
<td>0.685</td>
</tr>
<tr>
<td>% Married or living as married</td>
<td>79.5</td>
<td>64.3</td>
<td>79.3</td>
<td>71.9</td>
<td>75.0</td>
<td>66.7</td>
<td>0.294</td>
</tr>
<tr>
<td>MediCal³ (% yes)</td>
<td>28.2</td>
<td>42.9</td>
<td>31.0</td>
<td>36.8</td>
<td>33.3</td>
<td>33.3</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>WIC⁴ (% yes)</td>
<td>33.3</td>
<td>46.4</td>
<td>20.7</td>
<td>33.3</td>
<td>33.3</td>
<td>38.9</td>
<td>0.787</td>
</tr>
<tr>
<td>Current smoker (% yes)</td>
<td>5.1</td>
<td>3.6</td>
<td>10.3</td>
<td>7.0</td>
<td>6.3</td>
<td>11.1</td>
<td>0.610</td>
</tr>
</tbody>
</table>

¹P-value for the difference in baseline characteristics between those who completed follow-up and those who were lost to follow-up as analyzed by ANOVA for continuous variables and Fisher’s Exact test for categorical variables.
²Mean (SD) (all such values).
³California Medical Assistance Program - provides health coverage for people with low income.
⁴The Special Supplemental Nutrition Program for Women, Infants and Children - a federal assistance program for healthcare and nutrition of low-income pregnant women, breastfeeding women, and infants and children under the age of five.
⁵Fe-B, iron supplement consumed between meals; Fe-W, iron supplement consumed with meals
Table 2 Baseline and change from baseline Hb, transferrin saturation, and total plasma iron, 8-OHdG, isoprostane, TAS, CRP, AGP, AST and ALT.

<table>
<thead>
<tr>
<th>Time point</th>
<th>Placebo (n=38)</th>
<th>Fe-B (n=28)</th>
<th>Fe-W (n=29)</th>
<th>P Unadjusted</th>
<th>P Adjusted</th>
<th>Fe groups combined (n=57)</th>
<th>P Unadjusted</th>
<th>P Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>136 (11)</td>
<td>133 (11)</td>
<td>134 (11)</td>
<td>134 (11)</td>
<td>134 (11)</td>
<td></td>
<td>134 (11)</td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>-3.7 (12)</td>
<td>2.5 (9.6)</td>
<td>0.4 (11)</td>
<td>0.070</td>
<td>0.034^a</td>
<td>1.4 (10)</td>
<td>0.028</td>
<td>0.010</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>55.2 (38)</td>
<td>61.0 (47)</td>
<td>53.8 (38)</td>
<td>57.4 (42)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>-9.17 (34)</td>
<td>-13.1 (55)</td>
<td>6.12 (30)</td>
<td>0.088</td>
<td>0.091</td>
<td>-3.33 (44)</td>
<td>0.070</td>
<td>0.056</td>
</tr>
<tr>
<td>Hepcidin (ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>47.6 (35)</td>
<td>65.0 (67)</td>
<td>46.2 (32)</td>
<td>55.8 (53)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>18.8 (51)</td>
<td>34.2 (88)</td>
<td>42.2 (73)</td>
<td>0.700</td>
<td>0.028</td>
<td>38.2 (80)</td>
<td>0.406</td>
<td>0.139</td>
</tr>
<tr>
<td>Transferrin Saturation (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>22.3 (11)</td>
<td>19.6 (8.0)</td>
<td>19.6 (11)</td>
<td>19.6 (9.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>-0.78 (9.7)</td>
<td>6.13 (11)</td>
<td>1.62 (9.2)</td>
<td>0.145</td>
<td>0.087</td>
<td>3.84 (10)</td>
<td>0.111</td>
<td>0.271</td>
</tr>
<tr>
<td>Total plasma iron (µg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>89.6 (44)</td>
<td>78.1 (31)</td>
<td>77.2 (42)</td>
<td>77.6 (36)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>-15.8 (38)</td>
<td>10.5 (38)</td>
<td>-4.03 (42)</td>
<td>0.158</td>
<td>0.065</td>
<td>3.09 (40)</td>
<td>0.109</td>
<td>0.314</td>
</tr>
<tr>
<td>8-OHdG (ng/mg creatinine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>97.3 (37)</td>
<td>92.3 (36)</td>
<td>94.8 (39)</td>
<td>93.5 (37)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>-2.55 (43)</td>
<td>1.72 (51)</td>
<td>8.20 (57)</td>
<td>0.737</td>
<td>0.659</td>
<td>5.02 (54)</td>
<td>0.505</td>
<td>0.822</td>
</tr>
<tr>
<td>Isoprostane (pg/mg creatinine)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>366 (209)</td>
<td>406 (239)</td>
<td>377 (188)</td>
<td>392 (214)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>8.77 (126)</td>
<td>-3.80 (121)</td>
<td>-19.0 (131)</td>
<td>0.490</td>
<td>0.319</td>
<td>-11.5 (125)</td>
<td>0.403</td>
<td>0.226</td>
</tr>
<tr>
<td>TAS (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.69 (0.14)</td>
<td>1.69 (0.12)</td>
<td>1.65 (0.12)</td>
<td>1.67 (0.12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>-0.01 (0.11)</td>
<td>0.01 (0.09)</td>
<td>0.02 (0.11)</td>
<td>0.504</td>
<td>0.754</td>
<td>0.02 (0.10)</td>
<td>0.273</td>
<td>0.571</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>9.7 (9.8)</td>
<td>14.3 (25)</td>
<td>14.6 (19)</td>
<td>14.5 (22)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>-6.42 (11)</td>
<td>-8.89 (10)</td>
<td>-5.23 (12)</td>
<td>0.056</td>
<td>0.061</td>
<td>-7.0 (11)</td>
<td>0.610</td>
<td>0.642</td>
</tr>
<tr>
<td>AGP (g/L)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.2 (0.30)</td>
<td>1.3 (0.31)</td>
<td>1.3 (0.32)</td>
<td>1.3 (0.31)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>-0.33 (0.25)</td>
<td>-0.42 (0.23)</td>
<td>-0.28 (0.26)</td>
<td>0.237</td>
<td>0.094</td>
<td>-0.34 (0.25)</td>
<td>0.363</td>
<td>0.608</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>18.9 (6.0)</td>
<td>19.0 (7.1)</td>
<td>18.7 (6.9)</td>
<td>18.8 (7.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>0.37 (6.1)</td>
<td>2.07 (13)</td>
<td>0.69 (9.9)</td>
<td>0.656</td>
<td>0.489</td>
<td>1.37 (11)</td>
<td>0.458</td>
<td>0.367</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>14.5 (8.9)</td>
<td>5.7 (1.4)</td>
<td>13.1 (6.2)</td>
<td>13.1 (5.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>-2.34 (10)</td>
<td>0.93 (7.9)</td>
<td>-0.52 (8.5)</td>
<td>0.312</td>
<td>0.332</td>
<td>0.19 (8.2)</td>
<td>0.210</td>
<td>0.224</td>
</tr>
</tbody>
</table>

Change in values from baseline to final study visit. Data for ferritin, hepcidin, TfSat, plasma iron, 8-OHdG, isoprostane, CRP, and AGP were log transformed before statistical analyses of group-wise differences were performed.

Unadjusted difference across three randomization groups as evaluated by using ANOVA.

Differences across three randomization groups as evaluated by using ANCOVA. The baseline marker and covariates found to be significantly associated (P<0.10) with the outcome variable were included in the ANCOVA models. In addition to the baseline
value, hemoglobin was also adjusted for BMI and baseline ferritin, hepcidin, CRP, and TAS by including those variables in the model; hepcidin was also adjusted for total school years and baseline Hb and TAS; TfSat was adjusted for total school years and baseline Hb and CRP; total plasma iron was also adjusted for baseline Hb, CRP, and TAS; isoprostane was also adjusted for BMI, number of school years, and menstrual status at the final study visit; TAS was also adjusted for hemoglobin at baseline, CRP at baseline, and MediCal status (state-sponsored program that provides health coverage for people with low income); CRP was also adjusted for 8-OHdG at baseline, ferritin at baseline, BMI, number of school years, menstrual status at the final study visit; AST was also adjusted for ferritin at baseline; ALT was also adjusted for 8-OHdG at baseline and MediCal status.

Unadjusted difference between the iron groups combined and the placebo group as evaluated by ANOVA. The same adjustments were made as with the 3-group analysis as described above.

Placebo vs Fe-B, P=0.063; Placebo vs Fe-W, P=0.114

There was an interaction between group assignment and baseline CRP in the 2-group ferritin analyses. Interaction results are presented in Table 3.

Fe-B, iron supplement consumed between meals; Fe-W, iron supplement consumed with meals; Hb, hemoglobin; 8-OHdG, 8-hydroxy-2-deoxyguanosine; TAS, total antioxidant status; CRP, C-reactive protein; AGP, alpha-1-acid glycoprotein; AST, aspartate transaminase; ALT, alanine transaminase.
Table 3 Group-wise differences in change in ferritin from baseline as categorized by elevated and not elevated baseline CRP.

<table>
<thead>
<tr>
<th>CRP≤5 mg/L</th>
<th>Placebo (n=15)</th>
<th>Fe combined (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin (ng/mL)</td>
<td>-25.5 (29)</td>
<td>8.8 (26)</td>
</tr>
</tbody>
</table>

There was a significant interaction ($P=0.03$) between intervention group and baseline CRP for the change in ferritin. Data were log transformed before statistical analyses of comparisons between groups.

Differences between the iron groups combined and the placebo group as evaluated by ANCOVA after adjusting for baseline ferritin by including it as a covariate in the model.

CRP, C-reactive protein.
Table 4 Number and percentage of women with oxidative stress, ferritin, hepcidin, Hb, and inflammation values above or below cut-offs at the final study visit.

<table>
<thead>
<tr>
<th>Cut-off value</th>
<th>Placebo (Baseline: n=41; Final: n=38)</th>
<th>Fe-B (Baseline: n=37; Final: n=28)</th>
<th>Fe-W (Baseline: n=36; Final: n=29)</th>
<th>$P$ Adjusted</th>
<th>Fe groups combined (Baseline: n=73; Final: n=57)</th>
<th>$P$ Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hb (g/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;120</td>
<td>Baseline 3 (7) $^4$</td>
<td>7 (19)</td>
<td>2 (6)</td>
<td>9 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Final 1 (3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.996</td>
<td>0 (0)</td>
<td>0.954</td>
</tr>
<tr>
<td><strong>Ferritin (ng/mL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15</td>
<td>Baseline 3 (7)</td>
<td>4 (11)</td>
<td>5 (14)</td>
<td>9 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Final 5 (13)</td>
<td>1 (4)</td>
<td>2 (7)</td>
<td>0.194</td>
<td>3 (5)</td>
<td>0.070</td>
</tr>
<tr>
<td>&lt;30</td>
<td>Baseline 12 (29)</td>
<td>10 (27)</td>
<td>10 (28)</td>
<td>20 (27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Final 18 (46)</td>
<td>8 (29)</td>
<td>6 (21)</td>
<td>0.019$^4$</td>
<td>14 (25)</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Hepcidin (ng/mL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;8</td>
<td>Baseline 6 (15)</td>
<td>1 (3)</td>
<td>2 (6)</td>
<td>3 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Final 3 (8)</td>
<td>0 (0)</td>
<td>1 (3)</td>
<td>0.997</td>
<td>1 (2)</td>
<td>0.960</td>
</tr>
<tr>
<td>&lt;18</td>
<td>Baseline 9 (22)</td>
<td>7 (19)</td>
<td>7 (19)</td>
<td>14 (19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Final 4 (10)</td>
<td>1 (4)</td>
<td>2 (7)</td>
<td>0.549</td>
<td>3 (5)</td>
<td>0.409</td>
</tr>
<tr>
<td><strong>8-OHdG (ng/mg creatinine)</strong></td>
<td>&gt;1SD</td>
<td>8 (20)</td>
<td>6 (16)</td>
<td>5 (14)</td>
<td>11 (15)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Final 5 (13)</td>
<td>3 (11)</td>
<td>9 (31)</td>
<td>0.043$^4$</td>
<td>12 (21)</td>
<td>0.306</td>
</tr>
<tr>
<td><strong>Isoprostane (pg/mg creatinine)</strong></td>
<td>&gt;1SD</td>
<td>4 (10)</td>
<td>5 (14)</td>
<td>4 (11)</td>
<td>9 (12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Final 7 (18)</td>
<td>4 (14)</td>
<td>5 (17)</td>
<td>0.621</td>
<td>9 (16)</td>
<td>0.430</td>
</tr>
<tr>
<td><strong>CRP (mg/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;5</td>
<td>Baseline 23 (56)</td>
<td>23 (62)</td>
<td>20 (56)</td>
<td>43 (59)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Final 8 (21)</td>
<td>6 (21)</td>
<td>11 (38)</td>
<td>0.330</td>
<td>17 (30)</td>
<td>0.346</td>
</tr>
<tr>
<td><strong>AGP (g/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1</td>
<td>Baseline 31 (76)</td>
<td>31 (84)</td>
<td>27 (75)</td>
<td>58 (79)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Final 12 (31)</td>
<td>7 (25)</td>
<td>9 (31)</td>
<td>0.511</td>
<td>16 (28)</td>
<td>0.251</td>
</tr>
</tbody>
</table>

$^4$ Difference between three study groups in the proportion of women having values above or below the stated cut-off value adjusted for the baseline category (high or low) as determined by logistic regression modeling. 8-OHdG was also adjusted for high baseline CRP and AGP (categorical variables); isoprostane was also adjusted for BMI, total number of school years completed, high baseline CRP (categorical variable); ferritin was also adjusted for BMI; hepcidin was also adjusted for BMI and total number of school years completed; CRP was also adjusted for BMI and low baseline ferritin (categorical variable); AGP was also adjusted for BMI, menstrual status at the final study visit, and high baseline 8-OHdG (categorical variable). Adjustments were made by putting the covariates in the regression models.

$^5$ Difference between the iron groups combined and the placebo group in the proportion of women having values above or below the stated cut-off value adjusted for the baseline category (high or low).
Iron & Oxidative stress postpartum.

773 n (%) (all such values).
774 Fe-W vs. Placebo, P=0.147; Fe-B vs. Placebo, P=0.050 as determined by Poisson regression.
775 Fe-W vs. Placebo, P=0.086; Fe-W vs. Fe-B, P=0.062 as determined by Poisson regression.
776 Fe-B, iron supplement consumed between meals; Fe-W, iron supplement consumed with meals; 8-OHdG, 8-hydroxy-2-deoxyguanosine; Hb, hemoglobin; CRP, C-reactive protein; AGP, alpha-1-acid glycoprotein
Iron & Oxidative stress postpartum.

Enrollment

Assessed for eligibility (n=514)

Excluded (n=400)
- Not meeting inclusion criteria (n=141)
- Declined to participate (n=199)
- Could not contact to assess Hb (n=57)
- Hb < 110 g/L (n=3)

Randomized (n=114)

Allocation

Allocated to Fe-B (n=37)
- Received allocated intervention (n=37)

Allocated to Fe-W (n=36)
- Received allocated intervention (n=36)

Allocated to Placebo (n=41)
- Received allocated intervention (n=41)

Follow-Up

Lost to follow-up (n=2)
- Discontinued intervention (n=2)

Lost to follow-up (n=9)
- Discontinued intervention (n=3)

Lost to follow-up (n=7)
- Discontinued intervention (n=3)

Analysis

Analyzed (n=28)

Analyzed (n=39)

Analyzed (n=29)
Figure 1 Schematic representation of recruitment, enrollment, and follow-up. More women were lost to follow-up in the iron groups than in the placebo group ($P=0.048$ for the overall 3-group model; $P=0.01$ for Fe-B vs. Placebo; $P=0.047$ for Fe-W vs. Placebo; $P=0.02$ for the combined iron groups vs. Placebo). Fe-B, iron supplement consumed between meals; Fe-W, iron supplement consumed with meals.