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Early deterioration of iron status among a cohort of Bolivian infants

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Abstract

Iron deficiency (ID) and iron deficiency anemia (IDA) are major contributors to infant and maternal morbidity worldwide. There is limited longitudinal data on iron status in young infants and on methods to adjust iron biomarkers for inflammation. We aimed to quantify the prevalence of inflammation-adjusted ID, anemia, and IDA over the first year in a cohort of Bolivian infants and their mothers. Healthy mother-infant dyads were recruited from two peri-urban hospitals. Infants provided three blood draws (2, 6–8, and 12–18 months; $N = 160$); mothers provided two blood draws (1 and 6–8 months postpartum [plus third anemia measurement at 12–18 months]; $N = 250$). Blood was analyzed for hemoglobin, ferritin, soluble transferrin receptor, C-reactive protein (CRP), and alpha(1)-acid glycoprotein (AGP). Iron biomarkers were adjusted for inflammation using CRP and AGP; hemoglobin cutoffs were adjusted for altitude. Inflammation (elevated CRP or AGP) was 17% among toddlers 12–18 months of age. ID (inflammation-adjusted ferritin) increased with age (<1%, 56%, and 79% at each blood draw), as did anemia and IDA

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CONFLICTS OF INTEREST

Burke, Rebolledo, Fabiszewski de Aceituno, Revollo, Iñiguez, Klein, Drews-Botsch, Leon, Suchdev: no conflicts of interest. The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

CONTRIBUTIONS

PS, JL, CDB, MK, VI, AFdA, PAR, and RMB designed the research. PS, JS, AFdA, PAR, RR, VI, and RMB conducted the research. RMB analyzed the data. RMB, PS, and JS wrote the paper and had primary responsibility for final content. All authors have read and approved the final manuscript.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

(anemia: 70%, 76%, and 81%; IDA: <1%, 46%, and 68%). Maternal ID declined from the first to second assessment (39% vs. 27%). Inflammation-adjusted ID prevalence was up to 15 percentage points higher than unadjusted estimates. The high prevalence of ID, anemia, and IDA in this cohort of Bolivian infants beginning at 6–8 months of age suggests that early interventions may be necessary in vulnerable populations.

Keywords

anemia; global micronutrient malnutrition; infant nutrition; iron deficiency; iron deficiency anemia; micronutrient deficiencies

1 | INTRODUCTION

Iron deficiency (ID), which can progress into iron deficiency anemia (IDA) if uncorrected, is one of the most common micronutrient deficiencies globally: an estimated 40% of children under 5% and 38% of pregnant women worldwide suffer from ID (Camaschella, 2015). ID and IDA can have especially severe consequences in young children as well as pregnant women and have been associated with reduced cognitive development in infants, preterm delivery, and maternal death (Milman, 2011a, Ziegler, Nelson, & Jeter, 2011).

Infants are typically born with high stores of iron, which are accumulated during the third trimester of gestation and are thought to last for at least 4–6 months (Burke, Leon, & Suchdev, 2014). However, the size of these stores at birth can vary, and given the low intake of iron through breast milk in the first months of life, birth stores may be an important determinant of later risk of iron deficiency (Burke et al., 2014). Although multiple studies have gathered data on iron status on infants at different ages (Capozzi, Russo, Bertocco, Ferrara, & Ferrara, 2010; Domellof, Dewey, Lonnerdal, Cohen, & Hernell, 2002; Finkelstein, O'Brien, Abrams, & Zavaleta, 2013; Marques, Taddei, Lopez, & Braga, 2014; Michaelsen, Milman, & Samuelson, 1995; Olaya, Lawson, & Fewtrell, 2013; Preziosi et al., 1997; Sherriff, Emond, Hawkins, & Golding, 1999), few have provided data for healthy infants followed from breastfeeding age through complementary food introduction and up to 1 year of life (Hay et al., 2007; Willows, Dewailly, & Gray-Donald, 2000; Ziegler, Nelson, & Jeter, 2014). These published studies suggest that iron status declines significantly over the first year of life, leading to ID in vulnerable populations. Further, although ID cut-points have been suggested for young infants (<6 months), none have been universally agreed upon (Domellof et al., 2002; WHO, 2004). It may therefore be useful to assess cut-points in young, breastfeeding infants to predict later ID at the age of complementary food introduction and afterwards.

Similarly, mothers also undergo changes in iron metabolism during and following pregnancy, and increased blood volume can affect concentration of iron biomarkers (Milman, 2011b). Delivery itself can cause large blood losses, which also affect iron and hemoglobin concentration. Following pregnancy, mothers begin a process of returning to normal blood volume and composition, one aspect of which is recycling of hemoglobin iron to restore body iron stores. These processes are generally thought to normalize by 6–8 weeks postpartum (Milman, 2011b). However, although results of some studies have been

consistent with this hypothesis (van Santen et al., 2013), others have demonstrated that some changes may last past this time (Bjorke-Monsen, Torsvik, Ueland, Saetran, & Sandberg, 2012; Choi & Pai, 2001; Taylor, Mallen, McDougall, & Lind, 1982; Wallenburg & Van Eijk, 1984), suggesting that the duration of the "postpartum period" may be longer. This opens the possibility that ID cut-points for recently postpartum women should be different from those for nonpregnant women (Milman, 2011b), although no current guidelines exist (WHO, 2004). Additional data regarding changes in iron status biomarkers in postpartum women will inform policies regarding the timing and target population for postpartum iron supplementation.

Iron status and response to iron interventions can be assessed through multiple biomarkers, which are also used to diagnose ID and IDA (WHO, 2004). Ferritin (Fer), a sensitive measure of iron stores, is highly affected by inflammation (typically defined by elevated levels of the acute phase reactants C-reactive protein [CRP] and alpha(1)-acid glycoprotein [AGP]), even when the inflammation is subclinical (Thurnham & McCabe, 2012; Thurnham, Northrop-Clewes, & Knowles, 2015; Raiten et al., 2015). Soluble transferrin receptor (sTFR) and total body iron (TBI) are also affected, although to a lesser extent (Cook, Flowers, & Skikne, 2003; Raiten et al., 2015; Thurnham & McCabe, 2012; Thurnham et al., 2015). Therefore, it is critical, particularly in areas with high background levels of inflammation, to adjust iron biomarkers for the effect of inflammation (Raiten et al., 2015). Several different methods are typically considered for this adjustment, each with advantages and disadvantages, but no international guidelines exist (Raiten et al., 2015; Thurnham & McCabe, 2012). Adjusting for inflammation in high-inflammation areas, such as those with high malarial and HIV burden, may change estimates of ID by up to 30 percentage points, depending on the adjustment method used (Engle-Stone, Nankap, Ndjebayi, Erhardt, & Brown, 2013, Grant et al., 2012). However, there are limited data on how different inflammation-adjustment methods perform across populations with different ages, sociodemographics, background inflammation, and from different geographies. Further, most studies to date focus on high-inflammation settings.

Key messages

- Iron deficiency, anemia and iron deficiency anemia were highly prevalent in this cohort of high-altitude Bolivian infants, and increased through one year of age.
- Micronutrient supplementation programs in Bolivia may need to be complemented or strengthened to increase adherence and impact.
- Iron status estimates can be meaningfully affected by inflammation, and this effect should be accounted for interpretation of programmatic impact.

To address these needs, this study sought to quantify the prevalence of ID, anemia, and IDA at several time points during the first year of life, while adjusting for inflammation, in a cohort of mother-infant dyads in El Alto, Bolivia. A secondary objective was to investigate the predictive value of early iron status on later iron status among mothers and their infants. This high-altitude population, while under-resourced, does not suffer a high prevalence of

malaria or HIV and is thus an important comparator to past research on iron trajectory and adjustment for inflammation, given that prior studies were mainly in African settings where such diseases are endemic.

2 | PARTICIPANTS AND METHODS

2.1 | Study population and design

Data for this study were drawn from the *Nutrición, Inmunología, y Diarrea Infantil* study, the primary aim of which was to assess the effect of global nutritional status on response to the rotavirus vaccine (scheduled at 2 and 4 months of age). As part of other maternal and child health programming, Bolivian policies recommend iron drops for preterm infants aged 2–6 months and provide families with 60 sachets of multiple micronutrient powder (MNP; “Chispitas”) every 6 months during the 6–59-month age range (AIEPI, 2006). Mothers receive a 3-month supply of ferrous sulfate—folic acid tablets during pregnancy. The study setting of El Alto (altitude 4000 m) is a peri-urban locale home to a largely indigenous population, most of relatively low socioeconomic resources. Although iron-fortified infant formulas are available and utilized, iron-fortified cereals are rarely a part of complementary feeding in this population.

In brief, 461 healthy infants (2–4 weeks of age) and their mothers were recruited from two hospitals during well-child or vaccination visits. Exclusion criteria included infant acute illness, suspected immunodeficiency (e.g., HIV), congenital malformations, and maternal inability to speak and understand Spanish or Aymara. Recruitment took place from May 2013 through March 2014, and infant-mother dyads were followed for 12–18 months, with final data collected in March 2015. The study comprised seven hospital visits and two in-home visits, with blood drawn from mothers at a target schedule of 1 and 6–8 months postpartum and from infants at a target schedule of 2 months, 6–8 months, and (optionally) 12–18 months of age. Mothers were also offered anemia testing via finger stick at 12–18 months postpartum. The third infant blood draw, added to support a newly funded secondary aim, was approved only after 50% of infants had already completed the study; mothers were contacted to participate optionally in an extra visit and blood draw. To aid interpretation of trends over time, for the present analysis, only singleton infants with all three blood draws and mothers with three anemia measurements were included.

2.2 | Ethical approval

The protocol and instruments for this study were approved by the Emory University IRB (IRB00056127) and the Bolivian Comité de Etica de la Investigación (Research Ethics Committee). Mothers provided written informed consent in Spanish or Aymara after explaining the purpose of the study in their own words.

Mothers and infants were referred for anemia according to Bolivian guidelines (hemoglobin <13.7 g/dl for mothers, hemoglobin <10.9 g/dl for infants and toddlers; no altitude adjustment; AIEPI, 2006). Infants were also referred for stunting (length-for-age Z score < −2, as diagnosed by trained interviewers based on WHO growth charts) or wasting (weight-

for-length Z score < −2, as diagnosed by trained interviewers based on WHO growth charts; WHO, 2010).

2.3 | Laboratory analysis and definitions of anemia, iron deficiency, and inflammation

Venous blood was collected (1 ml) from mothers and infants using zinc-free syringes and tubes. Hemoglobin (Hb) was measured at point-of-care using a HemoCue® Hb (HemoCue America, Brea, CA, USA) system. Plasma was analyzed by sandwich ELISA for CRP (a marker of inflammation; limit of detection [LOD]: 0.5 mg/L), AGP (a marker of inflammation; LOD:0.1 g/L), Fer (LOD: 2 μg/L), and sTFRs(LOD0.5 mg/L;Ramco assay equivalents; VitMin Lab, Germany; Erhardt, Estes, Pfeiffer, Biesalski, & Craft, 2004). TBI was calculated from Fer and sTFR utilizing Cook's formula (Cook et al., 2003):

$$Total\ Body\ Iron\ (TBI) = -\frac{1}{0.1207} * \left(\left(\log_{10}\frac{sTFR}{Fer}\right) - 2.8229\right)$$

Hemoglobin cut-offs were adjusted for the high altitude (3500–4000 m) of El Alto and the surrounding area of La Paz (Sullivan, Mei, Grummer-Strawn, & Parvanta, 2008). Anemia was defined as Hb < 13.7 g/dl for infants and as Hb < 14.5 g/dl for mothers (two mothers that were pregnant at the last visit were excluded from analysis), based on WHO guidelines (WHO, 2008). Low iron stores were defined as Fer < 12 μg/L for infants and Fer < 15 μg/L for mothers (WHO, 2004). Values of sTFR > 8.3 mg/L were considered indicative of tissue ID for both mothers and infants. TBI was also used to assess ID, with a cut-off of TBI < 0 for both mothers and infants. Inflammation was defined as CRP > 5 mg/L or AGP > 1 g/L (Thurnham & McCabe, 2012; Thurnham et al., 2008).

2.4 | Adjustment of iron marker values

Given the well-known effect of inflammation on Fer, it was deemed important to adjust all iron marker values for CRP and AGP (Thurnham & McCabe, 2012). As in the Biomarkers Reflecting Inflammation and Nutrition Determinants of Anemia project (Suchdev et al., 2016), we explored various methods for accounting for inflammation: exclusion of inflamed individuals, calculation and use of correction factors (Thurnham & McCabe, 2012; Thurnham et al., 2010), and linear regression (Engle-Stone et al., 2013). Full results are presented in a supplementary table (supplementary Table 1). For the body of this paper, we present the crude (unadjusted) results and those adjusted with a linear regression method, chosen because it reflects the relationship of iron biomarkers to inflammation across the entire range of AGP and CRP. Briefly, the marker of iron status (log-transformed to meet normality assumptions) was modeled as a function of continuous CRP and AGP (also log-transformed to improve model fit):

$$\ln\left(Ferritin\right) = \beta_0 + \beta_1 \ln\left(CRP\right) + \beta_2 \ln\left(AGP\right).$$

Estimated coefficients of AGP and CRP were then used in the adjustment equation:

$$\ln(Fer_{corr}) = Fer_{obs} - \hat{\beta}_1(\ln(CRP_{obs}) - \ln(CRP_{ref})) - \hat{\beta}_2(\ln(AGP_{obs}) - \ln(AGP_{ref}))$$

For observations where neither CRP nor AGP exceeded the referents, ferritin was not corrected. For observations where only CRP was above the referent value, only CRP was used in the equation. For observations where only AGP was above the referent value, only AGP was used in the equation. As there are no established reference values for normal CRP and AGP in the literature, the first deciles of CRP and AGP in the non-inflamed population were used as the referent values for the reported results. Adjusted TBI was calculated by applying Cook's formula to the adjusted Fer and sTFR values.

2.5 | Statistical methods

To assess differences between continuous variables (e.g., Fer) at different time points, the Wilcoxon signed rank test was used given non-normal distributions and paired data. To assess differences between categorical variables (e.g., ID) at different time points, the McNemar test for paired data was used. Fisher's exact chi-square test was used to test unpaired categorical data. Alpha was set to .001 to correct for multiple comparisons. Cut-points were examined, and receiver operating characteristic (ROC) curves were plotted using the ROCR package in the R statistical computing environment (Sing, Sander, Beerenwinkel, & Lengauer, 2005). Reported cut-points were calculated to maximize accuracy while maintaining sensitivity of at least 60% and specificity of at least 35%. Data were cleaned and analyzed using SAS v9.4 (Cary, NC, USA) and the R Environment for Statistical Computing (R Core Team, 2015).

3 | RESULTS

3.1 | Study population

The study population included 160 singleton infants with data available from all three blood draws (Figure 1). For mothers, analyses include nonpregnant women with three anemia measures ($N = 250$, Figure 1). Infants were recruited at an average age of 1 month and were relatively evenly distributed in terms of gender (Table 1). Nearly one-third were born via Caesarian section, one-sixth preterm, and less than one-tenth low birth weight; fewer than one-fifth were born with a birth interval <36 months. Virtually all infants had been breastfed at some point in their life, and the majority received some form of micronutrient supplementation by the age of 1 year. Approximately half of the infants were first-born children; the average age of mothers was 25 years, the average maternal body mass index was 26, and most mothers were married or cohabiting with the infant's father. Based on maternal education and household characteristics, the study sample was of generally low socioeconomic status (Table 1).

3.2 | Feeding practices and global nutrition

Nearly all infants were partially breastfed for at least 6 months, with 83% still continuing to breastfeed after 1 year of age (Table 2). Exclusive breastfeeding was lower, with only 59% of infants exclusively breastfed at the first assessment of iron status (around 2 months of age). Stunting was prevalent in 15% of infants at the first blood draw, 12% at the second blood draw, and 20% at the last blood draw. Overweight varied across time points from 19% to 28%. Inflammation reached a maximum of 21% at 6–8 months of age. Maternal inflammation was highest (39%) in the first month after delivery and then significantly

decreased to 18% at the second blood draw. Anemia was present in most infants, with 81% classified as anemic by 1 year of age; however, the prevalence of anemia was not statistically significantly different by time point. Maternal anemia was highest 1 month after pregnancy (28%) and then decreased.

3.3 | Infant iron status

At the first blood draw (around 2 months), most infants still had birth iron reserves (as indicated by Fer and TBI above ID thresholds) and adequate tissue iron (measured by sTFR ≤ 8.3 mg/L). These stores declined significantly ($p < .0001$) by the second blood draw (around 6–8 months), with median inflammation-adjusted Fer falling from 149 to 10 μg/L and then declining further to 6 μg/L by the third blood draw (around 12–18 months of age; Table 3). Iron status also worsened over time as measured by sTFR and TBI. Because differences between inflammation-adjusted and unadjusted values were meaningful, results will primarily report adjusted values.

These temporal patterns of declining iron status were also reflected in prevalence estimates of ID (Table 4). By the second blood draw (around 6–8 months), 56% of infants had low iron stores, defined as inflammation-adjusted Fer < 12 μg/L (unadjusted ID was 40%). By the third blood draw (12–18 months), 79% (66% for unadjusted Fer) of infants had low iron stores. Low tissue iron, defined as inflammation-adjusted sTFR > 8.3 mg/L, also steadily rose in prevalence (27% at the third blood draw), as did ID as defined by inflammation-adjusted TBI < 0 (67% at the third blood draw). All differences between time periods were statistically significant ($p \leq .0001$), with the exception of tissue ID from the first to second blood draws.

Given that anemia was present in nearly all infants, the prevalence of IDA closely tracked the prevalence of ID (Table 4). Differences between time periods were again statistically significant with the exception of STFR-defined IDA from the first to the second blood draw.

Prevalence estimates for ID and IDA utilizing all available data (vs. only infants with all three blood draws) were very similar (within 3 percentage points) across visits, markers, and using inflammation-adjusted as well as non-adjusted values (data not shown). Prevalence estimates for anemia utilizing all available data were also within 3 percentage points of estimates using only infants with all three blood draws (data not shown).

3.4 | Maternal iron status

Mothers' iron status improved from the first blood draw (1 month postpartum) to the second blood draw (6–8 months postpartum). At the first visit, median inflammation-adjusted Fer was 19 μg/L, whereas at the second visit, median adjusted Fer was 25.9 μg/L (Table 3). Similarly, iron status as measured by sTFR and TBI also significantly improved.

Maternal ID decreased in the second blood draw as compared with the first blood draw. At the first blood draw (1 month postpartum), 39% of mothers had low iron stores as defined by adjusted Fer < 15 μg/L (Table 4). At the second blood draw (6–8 months postpartum), this prevalence dropped to 27%. Patterns for sTFR and TBI were similar (Table 4). Differences

in ID between the first and second blood draws did not reach our threshold for significance ($p < .0001$) but were meaningful.

As in infants, patterns of mothers' IDA also tracked their ID prevalence (Table 4). Adjusted estimates of IDA were not significantly different ($p < .0001$) by time period but approached significance for STFR.

Prevalence estimates for ID and IDA utilizing all available data (as opposed to only mothers with three anemia measurements; $N = 358$ for first two visits) were very similar (within 3–4 percentage points) across visits, markers, and using inflammation-adjusted as well as non-adjusted values (data not shown). Prevalence estimates for anemia utilizing all available data were also within 4 percentage points of estimates using only mothers with three anemia measurements (data not shown).

3.5 | Exploration of ferritin cut-points

To examine the utility of different potential Fer cut-offs in identifying infants and mothers at high risk of developing ID, we constructed ROC curves to assess Fer as a predictor of later ID. Because not all iron biomarkers may always be accompanied by inflammatory biomarkers used for adjustment, we examined both adjusted and unadjusted biomarkers. Unadjusted values are presented for ease of comparison to other studies. Unadjusted infant Fer at the first blood draw (approximately 2 months of age) was a fair and nearly good predictor (Kleinbaum et al., 2010) of infant ID at the second blood draw (approximately 6–8 months), with an area under the curve (AUC) of 80%. A threshold of 167 μg/L predicted later ID with 87% sensitivity and 65% specificity (Figure 2). Infant Fer at the first blood draw was not very predictive of ID at the third blood draw (12–18 months; AUC of 56%). However, Fer at the second blood draw was a fair predictor of ID at the third blood draw (AUC of 72%), with a threshold of 28 μg/L providing 76% sensitivity and 63% specificity. Maternal Fer was not a good predictor of infant ID at any time point (data not shown). Maternal Fer at the first blood draw was a fair predictor of maternal Fer at the second blood draw (AUC of 73%; threshold of 18 μg/L provided 62% sensitivity and 75% specificity; Figure 2). Results were very similar for curves using inflammation-adjusted Fer for infants and mothers.

4 | DISCUSSION

4.1 | Infant iron status

In this cohort of Bolivian infants, ID was extremely common early in life; by 6–8 months of age, approximately half of infants had low iron stores as defined by Fer or TBI, and by the age of 12–18 months, approximately three quarters of infants had low iron stores. Although tissue ID was less common, it too increased from infancy into young toddlerhood. The difference in prevalence estimates of low iron status based on Fer and TBI as opposed to STFR reflects known biological processes, wherein Fer is depleted first, before STFR rises in response (Burke et al., 2014). Anemia was also very common (>70%), and nearly all iron-deficient infants were also anemic, leading to a high prevalence of IDA (46% at 6–8 months and 68% by 12–18 months). At the first blood draw, <1% of anemic infants also suffered ID

(as defined by low Fer). However, by the second blood draw, 58% of anemia was associated with ID, and at the third blood draw, 83% of anemia was associated with ID. This suggests that ID is a primary cause of anemia in this population.

The general pattern of decreasing infant iron stores and increasing prevalence of ID is consistent with iron biology and other research. Even in well-resourced populations (e.g., USA, Denmark, and Norway), Fer has been shown to decrease from birth through at least 6 months of age, as birth iron stores run out faster than they are replenished through diet (Hay et al., 2007; Kling, Roberts, & Widness, 1998; Michaelsen et al., 1995; Ziegler et al., 2014). Nonetheless, the (Fer-defined) ID prevalence observed in our cohort was higher than that observed in other Latin American populations (32% in 5-month-old Peruvian infants (Finkelstein et al., 2013); 26% in 6-month-old Brazilian infants (Marques et al., 2014)), although comparable with some other developing-country settings (59% in Nigerian 6-month-olds (Preziosi et al., 1997)). Among Latin American countries in a recent review, Bolivia has the highest national prevalence of anemia among preschoolers (6–59-month-olds; 61%; Mujica-Coopman et al., 2015), and so it is perhaps not surprising that our study also showed an extremely high prevalence of anemia in infants, despite our adjustment for the high altitude of the study setting. Comparably high anemia prevalence has also been previously shown in low-resource Chinese infants and toddlers (51–60% of 6–17-month olds (Hipgrave et al., 2014; Luo et al., 2014)), whereas high IDA has also been found in Saudi Arabian infants (49% at 6–24 months of age; Al Hawsawi et al., 2015). Overall, these comparisons suggest that our population showed a high prevalence of ID, IDA, and anemia even among other low-resource populations.

The high prevalence of ID in our cohort is concerning, especially in the context of a national supplementation program that regularly provides iron-containing MNPs to all infants and children 6–59 months of age (60 sachets, dose of one daily, provided twice per year) (*AIEPI*, 2006). Given that >90% of our sample reported receiving MNPs by the age of 12 months, and over 75% of these reported some use of MNPs, it is possible that the dose or iron compound (12.5 mg per sachet as ferrous fumarate (*AIEPI*, 2006)) was not sufficient, or that the iron was not adequately absorbed due to inflammation, diet, or other factors (Moretti et al., 2015). However, an additional study would be needed to determine the most appropriate iron delivery in this population. Additionally, low maternal iron status during pregnancy and other factors could potentially contribute to low birth iron stores. Although preterm birth is a known risk factor for ID (Burke et al., 2014) and we had a high percentage of preterm infants in our cohort, we did not observe this association in our cohort except for tissue ID (measured by STFR) at the second blood draw (data not shown); this may be related to insufficient power or to misclassification of preterm status, as clinics calculated gestational age using inconsistent methods. However, only 22% of these infants received iron drops according to recommendations (data not shown). Although altitude, given the high hemoglobin requirements it engenders, may be related to the extremely high prevalence of anemia (Hipgrave et al., 2014), it is unclear whether it would also explain the high prevalence of ID, especially among infants (Cook, Boy, Flowers, & Daroca Mdel, 2005). Other potential explanations include inadequate dietary intake (although approximately one-third of infants received iron-fortified formula, iron-fortified cereal was rarely reported as a complementary food), frequent enteric infections leading to reduced iron absorption,

maternal factors, and sociodemographics (Burke et al., 2014). Further research in this cohort will better elucidate the role of these and other potential risk factors to provide information to improve the timing and content of ID-prevention interventions.

4.2 | Maternal iron status

Mothers exhibited the opposite pattern from infants; consistent with prior literature (Bjorke-Monsen et al., 2012; Milman, 2011b; van Santen et al., 2013), their iron status improved over time postpartum, even after adjusting for inflammation, from a prevalence of 39% ID at 1 month postpartum to a prevalence of 27% at 6–8 months postpartum. This is consistent with biological understanding that maternal iron measures recover postpartum, partly attributable to reversal of hemodilution (Milman, 2011b). Although Fer in three other studies decreased or remained the same postpartum, those results are not fully comparable to ours: Two studies showed this decrease in a study arm without prenatal iron supplementation as compared with a supplemented study arm, and one study took place in a WIC population in the United States; further, none adjusted for the effect of inflammation (Pehrsson, Moser-Veillon, Sims, Suitor, & Russek-Cohen, 2001; Taylor et al., 1982; Wallenburg & Van Eijk, 1984). Although iron supplementation during pregnancy was inconsistently adhered to in our cohort (57% reported taking at least 1 month), >80% reported taking at least some iron during pregnancy. Indeed, iron supplementation of at least 1 month during pregnancy was significantly associated with better iron status at the women's first visit (data not shown), consistent with other research (Pehrsson et al., 2001; Taylor et al., 1982, Wallenburg & Van Eijk, 1984).

4.3 | Adjustment for inflammation

Several previous studies of ID and adjustment for inflammation have taken place in African settings where up to 50% of children had inflammation and found that estimates of ID can vary by 8–30 percentage points depending on the exact adjustment method used (Engle-Stone et al., 2013; Grant et al., 2012). Inflammation adjustment is also considered important in recently postpartum women, because delivery is a known inflammatory process that can transiently increase Fer, potentially leading to underestimates of ID if unaccounted for (Lee et al., 2014). Even in this relatively low-inflammation population (<22% of infants), adjustment of iron biomarkers for CRP and AGP meaningfully affected most estimates of iron status in infants and mothers. While using Fer, ignoring inflammation tended to result in a prevalence of ID that was approximately 15 percentage points lower, a meaningful difference for making clinical and public health decisions, suggesting that inflammation should be accounted for even where not highly prevalent. Because prevalence estimates may vary by correction method and there is no international guidance on correction method (Suchdev et al., 2016), both adjusted and unadjusted prevalence estimates should be presented.

4.4 | Ferritin cut-offs to predict later ID

Although Fer cut-offs are well established for infants of at least 6 months of age, no cut-offs are universally accepted for younger infants, and it is believed that iron homeostasis may not be fully developed even at 9 months of age (Domellof et al., 2002). Nonetheless, it may be useful to identify young infants that should be followed for ID. ROC curves suggested that

infant Fer could adequately predict later ID, with better prediction in the short term (4–6 months) versus long term (10+ months), and that very high birth iron stores (Fer >160 μg/L) are needed in this population to maintain iron status through introduction of complementary foods. Although we could find no other studies with ROC analysis of ID in infants, our findings are in line with research by Ziegler et al. in U.S. infants: Of infants who developed ID before 6 months of age, the mean Fer at 1 month of age was 125 μg/L, much lower than the average of 242 μg/L in the full sample (Ziegler et al., 2014), again suggesting that birth stores must be high to avoid ID at the age where complementary foods are introduced.

4.5 | Strengths and limitations

This study has several strengths. We followed this cohort of Bolivian infants and their mothers over the course of a full year of life, with multiple time points measured. In addition, we used AGP and CRP to adjust iron biomarkers for the effect of inflammation, increasing the validity of our estimates. Further, our population of healthy, primarily breastfed infants in a setting of routine national supplementation and universal child healthcare allows us to assess the natural course of iron status in a healthy developing-country population. However, there are also several limitations to this study. There was some loss to follow up, and not all mothers agreed to the third blood draw for their infant, resulting in a smaller analytical sample (only infants with all three blood draws were included). Further, not all mothers completed all three anemia measurements, reducing the maternal analytical sample. However, the population with all three blood draws was highly similar to the population without the third blood draw in terms of demographics in addition to prevalence of ID, IDA, and anemia at baseline and at the second blood draw (data not shown). Overall, there was a low participation rate (35% of eligible mother–infant pairs were enrolled); although exact percentages are not available, major reasons for failure to enroll included a lack of interest (e.g., due to time constraints or refusal of blood draw) and an inability to follow up with interested mothers (e.g., faulty contact information). Although this raises the possibility that mothers and infants refusing blood draws (and therefore not enrolled) were somehow different from those enrolled, it is encouraging that the sociodemographics and birth characteristics of enrolled mothers and infants in this study were very similar to those in a pilot study in the same hospitals but not requiring blood draws (unpublished observations). It is also unknown whether ID would continue to increase if infants were followed past 1 year of age. These results may not be generalizable to settings with a high prevalence of inflammation or other causes of anemia (such as malaria or HIV). Anemia results may not be generalizable to lower-altitude settings, including other areas of Bolivia.

5 | CONCLUSIONS

The prevalence of ID and IDA was high in this cohort of Bolivian infants, developing early by 6 months of age and increasing through 12–18 months of age in the setting of a national MNP supplementation program. The high prevalence of ID in young infants is concerning given the potentially severe sequelae of ID and suggests a need for interventions that can be implemented in pregnant women or young infants, to improve iron status even before infants are weaned. For instance, improved program delivery of iron supplementation of pregnant

women (currently provided but inconsistently adhered to) or delayed cord clamping at birth (currently recommended by the Bolivian national standards [Atención Integrada al Continuo del Curso de la Vida] but inconsistently practiced) are needed. Further, there may be an opportunity to improve adherence to MNPs (or ensure appropriate usage) or to initiate supplementation earlier (e.g., in the form of iron drops). More up-to-date data is required on national coverage and intake of MNPs. This research also suggests that ID prevalence among young infants may be higher than expected in other developing-country settings, indicating a need for additional research into early interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AGP	(alpha(1)-acid glycoprotein)
CRP	C-reactive protein)
Fer	(Ferritin)
Hb	(hemoglobin)
ID	(iron deficiency)
IDA	(iron deficiency anemia)
MNP	(multiple micronutrient powder)
ROC	(receiver operating characteristic)
sTFR	(soluble transferrin receptor)

TBI (total body iron)

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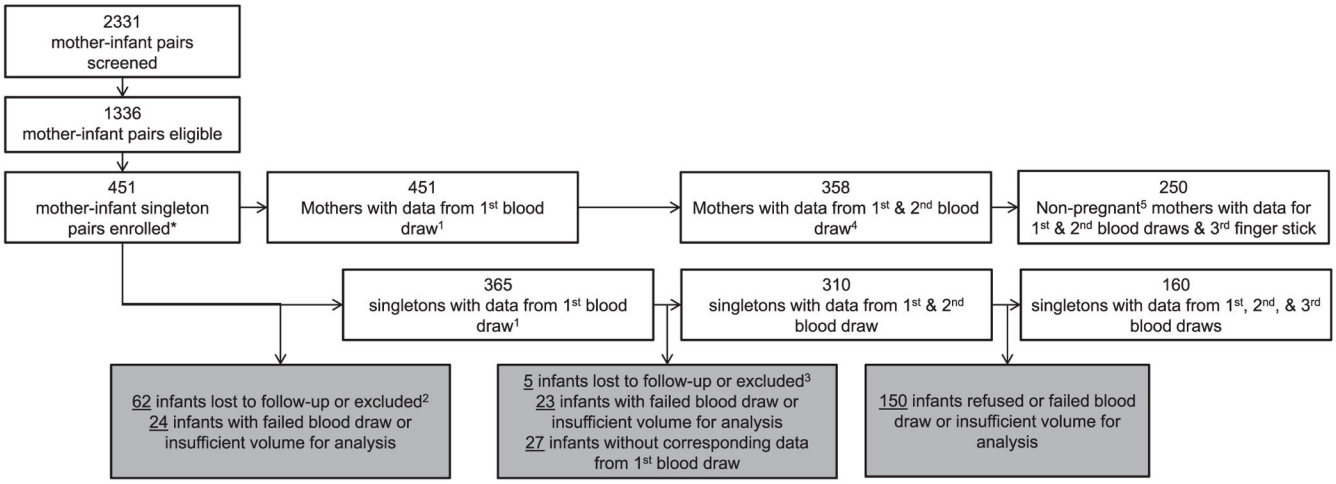

FIGURE 1.

Participant Flow. Of 2331 screened mother–infant pairs, 1336 were eligible and 451 singletons enrolled. A total of 365 singleton infants provided samples at 2 months, 310 at 2 and 6–8 months, and 160 at all three time points. [1]Maternal first blood draw at 1 month postpartum. Infant first blood draw at 2 months of age. [2]Main reasons for loss-to-follow-up and exclusion include failure to complete vaccines on schedule, missing required visits, and lost contact/participant moved out of study area. [3]Between first and second infant blood draws, infants excluded for lost contact. [4]Only two additional mothers failed their second blood draw. [5]At the last anemia assessment, two mothers were excluded for current pregnancy

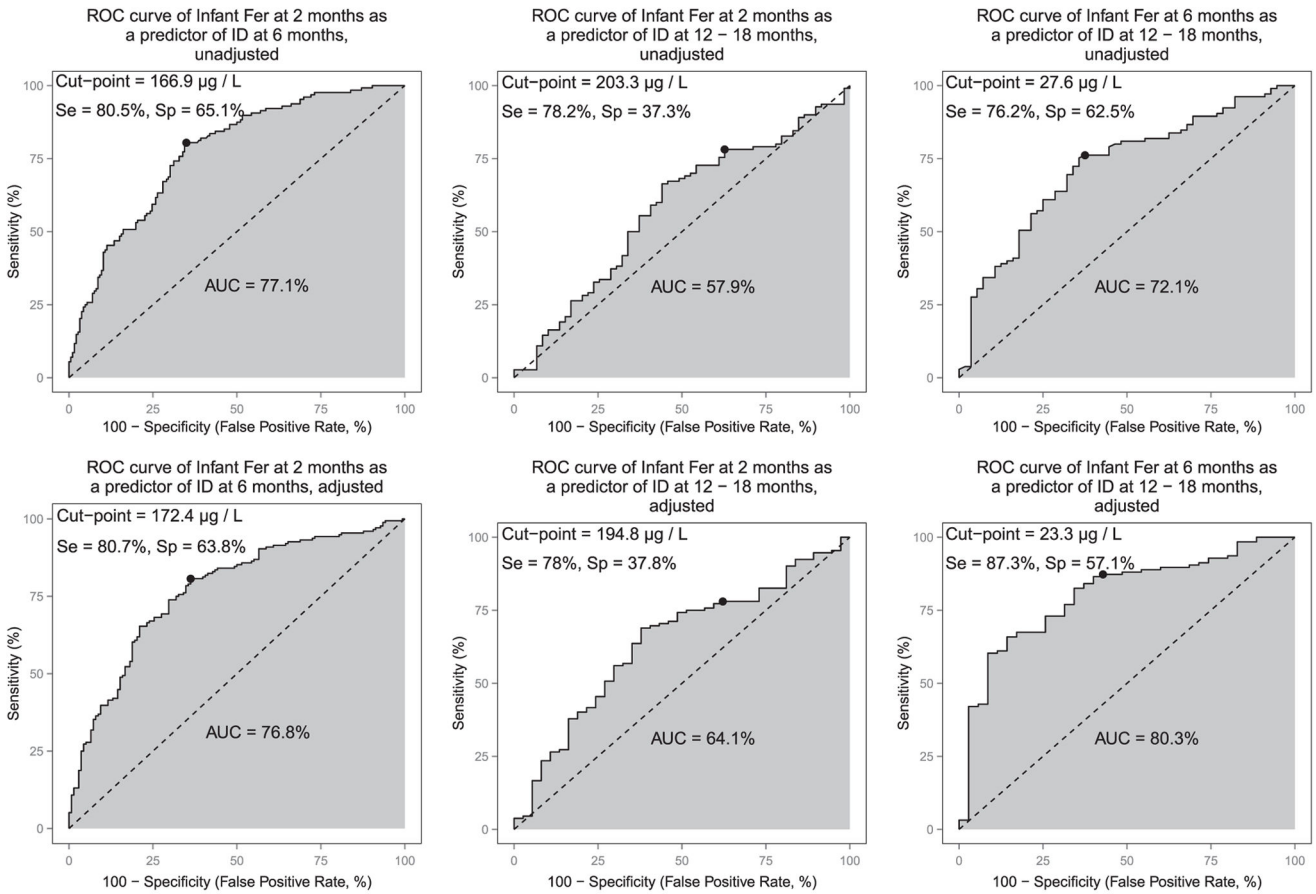

FIGURE 2.

ROC Curves of Ferritin and ID in Infants. Infant ferritin at 2 months of age is an adequate predictor of infant ID at 6–8 months of age but not of infant ID at 12–18 months. Cut-points for Fer at younger ages provide indicated Se and Sp for ID at later ages. The dashed line indicates no predictive value. AUC = area under the curve; Fer = ferritin; ID = iron deficiency; ROC = receiver operating characteristic; Se = sensitivity; Sp = specificity

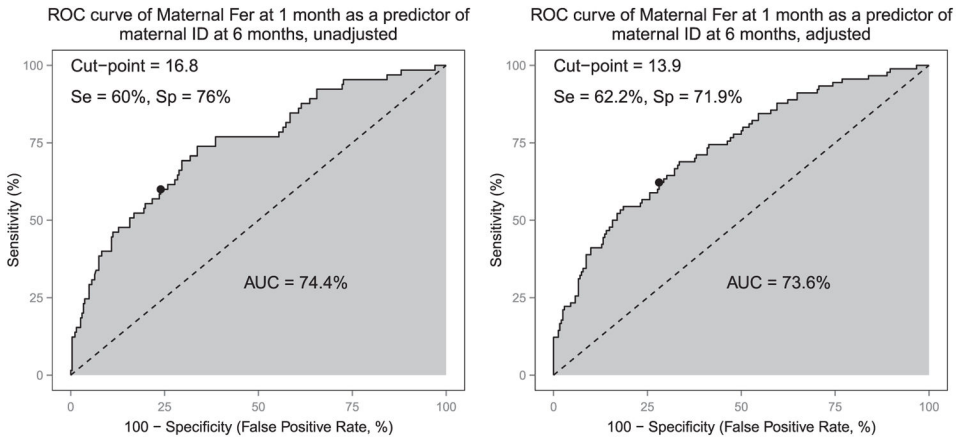

FIGURE 3.

ROC Curves of Ferritin and ID in mothers. Maternal ferritin at 1 month is an adequate predictor of maternal ID at 6–8 months postpartum, and prediction is not changed by adjustment for inflammation. Cut-points for Fer at 1 month provide indicated Se and Sp for ID at 6–8 months postpartum. The dashed line indicates no predictive value. AUC = area under the curve; Fer = ferritin; ID = iron deficiency; ROC = receiver operating characteristic; Se = sensitivity; Sp = specificity

TABLE 1

Characteristics of the study sample ($N = 160$)[a]

	Mean (±*SD*)	Frequency (*n*)	Percent (%)
Infant characteristics			
Age (days) at enrollment	33 ± 8	—	—
Male		85	53.1
C-section		48	30.0
Preterm (<37 weeks gestational age)		23	14.9
Low birth weight (<2500 g)		10	6.6
Birth interval <36 months (vs. ≥36 months or firstborn)		27	17.1
Took MNPs[b] by last visit		112	70.4
Took iron drops[c] by second blood draw		7	4.4
Maternal characteristics			
Primipara		125	50.0
Maternal age (years)	25.3 ± 6.3	—	—
Maternal BMI	26.3 ± 3.6	—	—
Took ≥1 month of prenatal iron supplementation		131	57.5
Sociodemographics			
Maternal Education			
Primary or less		41	15.6
At least some secondary		92	57.5
At least some superior		43	26.9
Other Sociodemographics			
Mother married or cohabitating		135	84.3
Maternal employment		41	25.8
Household size (number of people)	5.1 ± 2.3		—
Household owns refrigerator		40	25.0
Higher quality floor material (hardwood, carpet, or tile vs. cement)		54	33.8
Water piped indoors		65	40.6
Private toilet		90	60.0

[a] Of singleton infants with plasma available for all three blood draws. Maternal characteristics are given for mothers with three anemia measurements ($N = 250$).

[b] Multiple micronutrient powder sachets.

[c] Recommended for all preterm infants from 2 to 6 months of age. Of preterm infants, 13% ($N = 3$) took iron drops.

TABLE 2

Sample characteristics by age and time postpartum

	First blood draw[a] (range 1–5 months)		Second blood draw (range 6–10 months)		Third blood draw (range 10–19 months)	
	Mean (±*SD*) or frequency	**Percent**	**Mean (±*SD*) or frequency**	**Percent**	**Mean (±*SD*) or frequency**	**Percent**
N	160	—	160	—	160	—
Infant Age and Nutritional Characteristics						
Age (months)	2.1 ± 0.2	—	6.6 ± 0.7	—	14.1 ± 2.3	—
Exclusively breastfed[b]	93	58.5	15	9.4	0	0.0
Any breastfeeding	157	98.7	152	95.0	133	83.1
Stunted (LAZ < −2)	23	14.5	19	12.1	32	20.3
Overweight (WLZ > 1)	45	28.3	44	28.2	30	19.0
Inflammation[c]						
Infant	5	3.1	34	21.2	27	16.9
Maternal	98	39.2	46	18.4	N/A	—
Anemia						
Infant[†]	112	70.4	121	75.6	130	81.2
Maternal[d]	70	28.0	21	16.4	44	17.6

[a] 1-month postpartum for mothers (range 0.5–2 months). Maternal sample *N* = 250 includes all nonpregnant mothers of singletons, with three anemia measurements. All included infants must have data for all three blood draws.

[b] Exclusive breastfeeding is defined as infant is currently breastfeeding but has not received any non-breast milk liquids or semi-solid foods.

[c] Inflammation: CRP > 5 mg/L AGP > 1 g/L.

[†] Hemoglobin <13.7 g/dl (cut-off adjusted for altitude). Of anemic infants, <1%, 58%, and 83% were also ID (inflammation-adjusted Fer < 12 μg/L) at the first, second, and third blood draws, respectively.

[d] Hemoglobin <14.5 g/dl (cut-off adjusted for altitude). Of anemic mothers, 63% and 68% were also ID (inflammation-adjusted Fer < 15 μg/L) at the first and second blood draws, respectively.

TABLE 3

Maternal and infant iron status markers, adjusted* and unadjusted

	First blood draw[+] (range 1–5 months)		Second blood draw (range 6–10 months)		Third blood draw (range 10–19 months)	
	Median	**IQR (25%, 75%)**	**Median**	**IQR (25%, 75%)**	**Median**	**IQR (25%, 75%)**
N[b]	160	—	160	—	160	—
Ferritin (μg/L)						
Infant						
Unadjusted	156.0	(100.0, 203.9)	17.0[†]	(7.8, 38.2)	8.5[†‡]	(4.4, 16.3)
Adjusted[a]	149.1	(96.1, 194.2)	10.3[†]	(5.1, 20.5)	6.2[†‡]	(3.2, 10.6)
Maternal						
Unadjusted	27.5	(15.4, 49.7)	33.5	(17.7, 46.2)	N/A	—
Adjusted[a]	19.3	(10.4, 33.7)	25.9	(14.3, 34.8)	N/A	—
sTFR (mg/L)						
Infant						
Unadjusted	4.0	(3.4, 4.9)	5.3[†]	(4.6, 6.4)	6.6[†‡]	(5.3, 8.3)
Adjusted[a]	3.7	(3.2, 4.5)	5.2[†]	(4.4, 6.2)	6.5[†‡]	(5.4, 8.5)
Maternal						
Unadjusted	5.3	(4.1, 6.5)	4.3[†]	(3.6, 5.5)	N/A	—
Adjusted[a]	5.4	(4.2, 6.6)	4.4[†]	(3.6, 5.4)	N/A	—
TBI (mg/kg)						
Infant						

	First blood draw[+] (range 1–5 months)		Second blood draw (range 6–10 months)		Third blood draw (range 10–19 months)	
	Median	**IQR (25%, 75%)**	**Median**	**IQR (25%, 75%)**	**Median**	**IQR (25%, 75%)**
Unadjusted	11.8	(9.9, 12.8)	2.7[†]	(−0.5, 6.1)	−0.7[†‡]	(−3.7, 2.2)
Adjusted[a]	11.9	(10.0, 12.9)	1.4[†]	(−2.2, 4.0)	−1.8[†]	(−5.1, 0.7)
Maternal						
Unadjusted	4.4	(1.7, 7.3)	5.6	(3.3, 7.4)	N/A	—
Adjusted[a]	3.2	(0.3, 5.5)	4.6[†]	(2.8, 6.5)	N/A	—

[a]Linear regression method used, with first decile values in uninflamed population as inflammatory marker referents. Infant referents—first blood draw: CRP = 0.10 mg/L, AGP = 0.18 g/L; second blood draw: CRP = 0.12 mg/L, AGP = 0.29 g/L; third blood draw: CRP = 0.14 mg/L, AGP = 0.31 g/L. Maternal referents—first blood draw: CRP = 0.44 mg/L, AGP = 0.49 g/L; second blood draw: CRP = 0.32 mg/L, AGP = 0.46 g/L. TBI adjusted by applying Cook's formula to adjusted Ferritin and sTFR.

[b]Maternal sample *N* = 250 includes all nonpregnant mothers of singletons, with three anemia measurements.

[c]1 month postpartum for mothers (range 0.5–2 months).

[†]Significantly different ($p < .0001$) from 2- or 1-month value; Wilcoxon signed-rank test.

[d]Significantly different ($p < .0001$) from 6- to 8-month value; Wilcoxon signed-rank test.

TABLE 4

Prevalence (%) of maternal and infant iron deficiency (ID) and iron deficiency anemia (IDA) by iron status marker and time point, adjusted[b] and unadjusted

	First blood draw[a]		Second blood draw		Third blood draw	
	ID	**IDA**	**ID**	**IDA**	**ID**	**IDA**
N	160	—	160	—	160	—
Ferritin (μg/L)						
Infant						
Unadjusted	0.5	0.5	40.3[c]	34.8[c]	65.5[c,d]	57.5[c,d]
Adjusted[b]	0.5	0.5	55.5[c]	46.1[c]	79.2[c,d]	67.7[c,d]
Maternal						
Unadjusted	23.6	11.2	18.8	10.0	N/A	N/A
Adjusted[b]	38.8	17.6	26.8	11.2	N/A	N/A
STFR (mg/L)						
Infant						
Unadjusted	1.4	0.8	8.4	8.1	24.4[c,d]	22.8[c,d]
Adjusted[b]	0.8	0.5	7.4	7.1	26.8[c,d]	25.1[c,d]
Maternal						
Unadjusted	10.4	5.2	1.6	0.8	N/A	N/A
Adjusted[b]	10.4	5.2	2.0	0.8	N/A	N/A
TBI (mg/kg)						
Infant						

	First blood draw[a]		Second blood draw		Third blood draw	
	ID	IDA	ID	IDA	ID	IDA
Unadjusted	0.3	0.3	27.7[c]	25.8[c]	54.8[c,d]	52.1[c,d]
Adjusted[b]	0.3	0.3	39.0[c]	35.5[c]	67.3[c,d]	61.1[c,d]
Maternal						
Unadjusted	14.4	7.2	7.2	4.4	N/A	N/A
Adjusted[b]	22.0	11.2	10.8	6.8	N/A	N/A

[a] 1 month postpartum for mothers.

[b] Linear regression method used, with first decile values as inflammatory marker referents. Infant referents—first blood draw: CRP = 0.10 mg/L, AGP = 0.18 g/L; second blood draw: CRP = 0.12 mg/L, AGP = 0.29 g/L; third blood draw: CRP = 0.14 mg/L, AGP = 0.31 g/L. Maternal referents—first blood draw: CRP = 0.44 mg/L, AGP = 0.49 g/L; second blood draw: CRP = 0.32 mg/L, AGP = 0.46 g/L. TBI adjusted by applying Cook's formula to adjusted Ferritin and sTFR.

[c] Significantly different ($p < .0001$) from 2- or 1-month value; Wilcoxon signed-rank test.

[d] Significantly different ($p < .0001$) from 6- to 8-month value; Wilcoxon signed-rank test.