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Red blood cell metallothionein as an indicator of zinc status during pregnancy RBC metallothionein, zinc status and pregnancy

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Abstract

Objective—to describe the levels and patterns of change in red blood cell (RBC) metallothionein (MT) during pregnancy and the neonate, and relate RBCMT to other indicators of zinc and iron status.

Research Methods & Procedures—As part of a double-masked controlled trial of prenatal zinc supplementation among 242 Peruvian pregnant women, we determined RBCMT at enrollment (10–16 wk), 28 and 36 wk gestation, and in the cord blood at delivery in 158 women (86 who received daily supplements containing 60 mg iron and 250 ug folic acid, and 72 whose supplements also contained 25 mg zinc). In addition we measured plasma and urinary zinc concentrations, and hemoglobin and serum ferritin, and on a limited sample, we measured RBC zinc and placental MT.

Results—RBCMT increased during pregnancy, and levels in the cord blood approximated maternal values at 36 wk. Only RBC zinc at 36 wk differed by supplement type ($P < 0.05$). Increases in RBCMT over pregnancy were however, related to early pregnancy RBC zinc and inversely with the decline in plasma zinc from baseline to 36 weeks gestation.

Conclusion—Changes in RBCMT throughout pregnancy were consistent with the hypothesized role of MT in regulating zinc homeostasis. RBCMT appears to not be responsive during pregnancy to changes in zinc status achieved with supplements.

Keywords

Zinc; iron; metallothionein; erythrocyte; pregnancy

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INTRODUCTION

The public health importance of maternal zinc deficiency is increasingly recognized [1–3]. Yet, efforts to define the magnitude and distribution of zinc deficiency are hampered by a lack of indicators of zinc status among individuals and populations [4]. In the last 15 years there has been considerable interest in the concentration of metallothionein (MT) (a low-molecular weight, cysteine-rich, zinc-binding protein) in red blood cells (RBC), as a potential indicator of zinc status in different populations [5–8] including pregnant women [9–11]. In young men, RBCMT was shown to respond to zinc intake across a considerable range, including intakes at or below the current adult male RDA of 15 mg/d [5–8]. In women at late pregnancy, RBCMT was significantly higher than in non-pregnant women with similar dietary zinc intakes, and did not correlate with other biochemical zinc measurements [9,10]. Therefore, the significance of RBCMT as an indicator of zinc status during pregnancy remains unclear.

As part of a zinc supplementation trial in Peru, we investigated the usefulness of RBCMT as an indicator of zinc status in 158 women-neonate dyads, and its responsiveness to alterations in zinc status achieved with zinc supplements. The goals of the present paper are to describe the levels and patterns of change in RBC metallothionein during pregnancy and the neonate, and relate RBCMT to other indicators of zinc and iron status.

MATERIALS AND METHODS

Between 1998 and 2000, we conducted a double-masked controlled trial of prenatal zinc supplementation to improve fetal neurobehavioral development and growth among 242 Peruvian pregnant women. We conducted the study at the Hospital Materno Infantil San Jose in Villa El Salvador, an impoverished peripheral district in Lima, Peru, where pregnant women's zinc dietary intake is approximately 8 mg/d [12]. Previously, we have shown that in this population plasma and urinary zinc concentrations are lower than those observed in more zinc-replete populations and are responsive to zinc supplementation [13]. The overall study design and methods are described in full elsewhere [14,15], and only details relevant to the paper are described below. All women who participated in the study signed an informed consent form and the Institutional Review Boards of the Instituto de Investigación Nutricional and The Johns Hopkins Bloomberg School of Public Health approved the study protocol.

Women were eligible for the study if they were considered low risk (eligible for vaginal delivery), carrying a singleton fetus, and living in coastal Peru for at least six months before becoming pregnant. At entry into prenatal care, between 10 and 16 weeks gestation, women were randomly assigned in blocks of two, according to computer-generated randomization lists within parity and week of gestation at enrollment strata, to receive a daily supplement containing 60 mg iron (ferrous sulfate) and 250 μg folic acid, with or without 25 mg zinc (zinc sulfate). Gestational age was determined by last menstrual period and confirmed by ultrasonography at enrollment. The supplements were produced locally and had the same appearance and taste and were distributed in blister packs at monthly intervals. Supplementation began at entry into the study and continued until one month postpartum. Adherence with the treatment was checked biweekly by health workers who visited women in their homes and observed the number of tablets remaining in each blister pack. Women were queried regarding potential benefits, side effects, or problems which could be related to the supplements. Overall, about 90% of women consumed their supplements at least 5 days out of 7 [14], and this was true for the subsample of women studied here, with median compliances of 86% and 89% (of days) for the groups receiving, or not receiving zinc, respectively.

At baseline, women were interviewed to collect socio-demographic and other background information. At monthly intervals, actocardiography was used to record patterns of fetal heart

rate and fetal movement as indices of fetal neurobehavioral development, and ultrasonography was used to assess fetal growth. At enrollment, 28 weeks and 36 weeks gestation, we assessed maternal anthropometric status, and took blood and urine samples for mineral analyses. At each time point, we assessed hematocrit and concentrations of hemoglobin, albumin, plasma zinc, plasma ferritin, red blood cell metallothionein (RBCMT), and RBC zinc (on a limited number of samples because of cost). From the urinary samples, we assessed urinary zinc and creatinine concentrations. At birth, a sample of cord vein blood was obtained to assess plasma zinc, hemoglobin, plasma ferritin and RBCMT concentrations, and placental tissue was collected to assess placental MT (on a limited number of samples because of cost). Birth weight, length, and head circumference were assessed by hospital personnel.

Of the 242 women originally enrolled in the supplementation trial, 195 were in the final analytic sample to study fetal development outcomes, with losses to follow up due to miscarriage, fetal anomaly and attrition as detailed previously [14,15]. The data presented here pertain to the 158 women-neonate dyads with RBCMT data. Of these, 72 received zinc supplements and 86 did not; there were no differences in characteristics between those with or without RBCMT data. Based on a sample size of approximately 70 per group, we are able to detect differences on the order of 0.5 SD by treatment group. For RBC zinc and placental MT with sample sizes on the order of 15, detectable differences are on the order of 1 SD.

Blood samples were drawn by venipuncture into tubes containing heparin, and the samples were centrifuged at 600 g for 10 min for separation of plasma. The RBC were washed with ice-cold 0.9% NaCl and aliquots of RBC lysates were stored at −20 °C (6 to 12 months) until analyzed. Placentas were obtained at delivery and immediately processed as previously described [9]. Briefly, umbilical cord and amniotic membranes were removed; a tissue sample (~100g) was cut into small pieces, thoroughly washed with ice-cold 100 mM $CaCl_2$, followed by ice-cold 150 mM NaCL, for removal of contaminating blood; and, portions were stored at −20 °C (6 to 12 months) until analyzed.

RBC zinc was measured by atomic absorption spectrophotometry after overnight nitric acid (Suprapur-Merck) digestion of the samples. Results of RBC zinc were expressed per g of protein. The protein of the RBC lysate was measured by the Lowry method. The concentrations of MT in RBC and placenta were assayed using an adaptation of the ^{109}Cd-hemoglobin affinity assay of Eaton and Toal [16] as previously described [10]. This assay gives similar results for fresh and frozen samples [17].

Briefly, carrier-free ^{109}Cd (Amersham International, UK) was combined with $CdCl_2$ in 10 mM Tris-HCl, pH 7.4, to a final concentration of 5.0 or 10 μg of Cd/mL and final radioactivity of 0.1 or 0.2 μCi/mL, for determination in lysed RBC or cytosolic fraction of placentas, respectively. The RBC lysates were diluted three times with 10 mM Tris-HCl, pH 7.4, heated for 5 min in a water bath (at 100 °C), and centrifuged twice at 10000 g for 5 min. Cytosolic fractions of individual placentas were prepared from 10–20 g of tissue, thoroughly homogenized with 3 volumes of 10 mM Tris-HCl, pH 7.4, and centrifuged at 40,000 g for 10 min at 4 °C. The supernatants were heated in a water bath (at 100 °C) for 5 min and re-centrifuged under the same conditions for 30 min. Equal volumes of supernatant of heat-treated samples and ^{109}Cd solution were mixed and incubated for 10 min at room temperature. Unbound Cd was removed by two cycles of precipitation with 2% bovine hemoglobin followed by heat denaturation for 2 min, cooling on ice, and centrifugation at 10,000 g for 3 min. Radioactivity of equivalent aliquots of samples and appropriate blanks and standards were measured in a gamma-counter. The MT concentrations were calculated assuming that 6 g-atoms of Cd are bound per mol of MT. RBCMT was expressed in nmol MT/g protein and placental MT in nmol MT/g tissue. The intra-assay coefficients of variation (CV) for RBCMT and placental MT against a pooled reference sample were 5.8% and 6.9%, respectively.

Plasma and urinary zinc concentrations were measured using atomic absorption spectrophotometry (AAS, Perkin Elmer model 3100), and serum ferritin was measured by ELISA using reagents purchased from DAKO (Santa Barbara, CA). Bovine liver from the National Institute of Standards and Technology (Gaithursburg, MD) and ferritin controls (Diagnostic Products Corporation, Los Angeles, CA) were used as standards, and values were within expected ranges. Hemoglobin concentration was determined at the hospital using the cyanomethemoglobin method under the supervision of IIN laboratory staff.

We applied t-test to continuous variables and χ^2 test to categorical variables to examine comparability in maternal and newborn characteristics between supplement groups. To determine relationships between biochemical parameters we used Pearson's and Spearmans's correlations based on data distribution. Repeated measures analysis of variance (ANOVA) was performed to test changes in biochemical indicators across time, effect of supplement and interaction of supplement by time. Statistical analysis was fulfilled by using SAS version 9 (SAS Institute Inc., Cary, NC), with significance level of 0.05.

RESULTS

Maternal baseline characteristics, duration of pregnancy, and neonatal size at birth did not differ statistically by supplement type as presented in Table 1 and Table 2. These results are consistent with those reported elsewhere on the full analytic sample of 195 mothers and newborns.

Indicators of zinc and iron status throughout pregnancy and in the neonate are presented in Table 3. In general, there were no significant effects of supplement type on the zinc and iron indicators during pregnancy, but RBC zinc was higher in late pregnancy (36 wk) in the zinc-supplemented group ($P<0.04$; bivariate analysis). In both groups, plasma zinc declined over pregnancy ($P<0.001$), and urinary zinc excretion declined from 12 to 28 wk gestation ($P<0.001$), stabilizing from 28 to 36 wk gestation, consistent with previous studies [18,19, 11]. RBCMT increased slightly (on average by 9%) but significantly ($P<0.005$) during pregnancy, as previously observed [9]. RBCMT levels in cord blood approximated maternal values at the end of pregnancy, also as previously observed [10]. Placental MT concentration, on average 0.72 nmol/g, was much higher than observed in a previous study (0.30 nmol/g) [10]. As expected, hemoglobin and hematocrit declined from 12 to 28 wk gestation and then rose toward the end of pregnancy; ferritin concentrations declined and then stabilized. Also, ferritin levels in the neonate (cord blood) were much higher than values in the mothers at any stage of pregnancy. The overall patterns of changes in hemoglobin/hematocrit and ferritin were consistent with what would be expected for iron status during pregnancy in women who received supplemental iron [20].

Although there was pattern of cross-sectional differences in zinc and iron indicators during pregnancy by type of prenatal supplement, the decrease during pregnancy (from 12 to 36 wk) in urinary zinc was lower in the group receiving supplemental zinc compared with no zinc ($P < 0.02$). This supplement X type interaction was possibly due to a lower urinary zinc at early pregnancy (12 wk) in the zinc group compared with the non-zinc group ($P=0.047$; bivariate analysis). For the other indicators, there were no significant differences by group at baseline, or interactions between changes during pregnancy and type of supplement.

Shown in Table 4 are the correlations between the various biochemical indicators over time. Most parameters showed stability over pregnancy with correlations on the order of 0.3 to 0.6. The lowest correlation was for urinary zinc excretion between 12 and 36 wk gestation ($r = 0.19$). There were differences in the magnitude of the correlations between maternal and cord values across parameters, with the correlations being relatively strong for RBCMT and RBC

zinc, but negligible for plasma zinc and plasma ferritin concentrations. The correlations between MT in the cord, RBC, and the placenta were on the order of 0.25, but were not statistically significant, most likely due to the small sample size. The correlations between cord MT and placenta MT with other zinc and iron indicators during pregnancy were mostly negligible and non significant, except that placenta MT was negatively correlated with maternal hemoglobin (and hematocrit) at 36 wk ($r <= -0.37$).

We also examined the correlations between RBCMT concentrations (and changes) during pregnancy, and those of other indicators of zinc and iron status, and in the cord and the placenta (Table 5). RBCMT in late pregnancy (36 wk) correlated negatively with plasma zinc, albumin and ferritin in early pregnancy (12 wk), but positively (r=0.42) with RBC zinc. Moreover, the increase in RBCMT during pregnancy (from 12 to 36 wk) was positively correlated with RBC zinc in early pregnancy (12 wk) and negatively correlated with the decrease in plasma zinc from 12 to 36 wk (r=0.20) meaning that those with the smallest decline in plasma zinc had the greatest increases in RBCMT during pregnancy.

DISCUSSION

Maternal zinc status during pregnancy has been typically assessed using plasma zinc although it is recognized that this indicator may be poorly informative of zinc status due to efficient homeostatic regulation [4]. Because women do not typically increase their zinc intake during pregnancy [21], their increased zinc needs may be met mostly by physiological adjustments including increased efficiency of zinc absorption, reduced zinc excretion (including endogenous fecal excretion), and redistribution of tissue zinc [18,22,11]. In general, plasma zinc and other biochemical zinc indices during pregnancy reflect these physiological adjustments rather than maternal zinc status, thus justifying the continual search for new specific and sensitive indicators of nutritional status.

Based on studies in adults [5–8], and in women in late pregnancy [9,10], RBCMT appeared as a promising indicator of zinc status during pregnancy. However, before this measure can be used for this purpose, the pattern of change of RBCMT during pregnancy and its response to increased zinc intake needs to be evaluated.

In this study, in a Peruvian population with a typically low zinc dietary intake (8 mg/d), RBCMT increased during pregnancy but did not respond to the addition of 25 mg zinc to the daily prenatal supplement routinely used during pregnancy. The increase in RBCMT during pregnancy was modest (9%), considering that during the 168d period of the study (from 12 to 36wk of pregnancy), 140% of the initial RBC mass was replaced [fraction of initial RBC mass replaced: (1/120)× 168= 1.4]. This is in contrast to the fast and high responsiveness of RBCMT to zinc supplementation in adult men [8]. The increase in RBCMT during pregnancy in our study was negatively related to plasma zinc and plasma ferritin, and positively related to RBC zinc, at early pregnancy, suggesting that maternal zinc and iron status in early pregnancy may be important determinants of the change in RBCMT during pregnancy.

The increase in RBCMT during pregnancy appears to reflect a normal physiological adjustment of this state. Although the biological functions of MT have not yet been clearly defined [22], possible functions include regulation of intracellular zinc metabolism, acquisition and storage, [24] systemic zinc distribution in response to stress conditions and hormones [23], and protection against toxic metals and oxidation stress [25,26]. Therefore, the increase in RBCMT during pregnancy could reflect the increased metabolic demand for zinc to support the rapid proliferation and differentiation of red blood cells due to enhanced erythropoiesis. In addition, the increased RBCMT may ensure the increased activity during pregnancy of the zinc-dependent red blood cell enzyme carbonic anhydrase [27], and may protect maternal red blood

cells from the oxidation stress associated with the increased oxygen demand of pregnancy, acting as a free radical scavenger.

Since RBCMT was not influenced by zinc supplementation in this study, measurement of RBCMT may not be a sensitive index of zinc status during pregnancy. However, it should be noted that RBCMT in late pregnancy in this study (on average, 1.8 nmol/g protein) was ~34% lower than the average value observed at delivery (using the same analytical method with the same degree of measurement error), in pregnant women with higher dietary zinc intakes (13 mg/d) [9], suggesting some degree of association between RBCMT and maternal exposure to zinc. Moreover, our results indicate that the magnitude of RBCMT increase during pregnancy is possibly related to physiological adjustments of zinc and iron homeostasis at early pregnancy. Based on significant correlation coefficients, the increase in RBCMT during pregnancy was more pronounced in those women with a poorer zinc and iron status at early pregnancy (as indicated by lower plasma zinc and lower plasma ferritin, respectively), but also in those women with a higher RBC zinc at early pregnancy. RBCMT at late pregnancy was significantly correlated with RBCMT at earlier stages of pregnancy and the increase in RBCMT was associated with less decline in plasma zinc over pregnancy. Taken together, these results are consistent with the regulatory role of RBCMT in the acquisition of zinc by the red cells during pregnancy, and with a response possibly limited by maternal zinc status.

Based on the significant correlations observed in this study between maternal and cord zinc indices, RBCMT and particularly RBC zinc, it appears that maternal red blood cell zinc status influences the correspondent neonatal status, consistent with previous studies [28,10]. It should be noted that in our study both cord RBCMT and cord RBC zinc correlated with the corresponding maternal index at all stages of pregnancy, particularly at 12 wk, consistent with the hypothesis that the maternal zinc status at early pregnancy is an important determinant of the neonatal zinc status. This is in contrast to previous findings on iron from a study using stable isotopes, which suggested that iron transfer to the fetus in late pregnancy is regulated by status at the level of gut [30].

Measurement of placental MT may provide additional information on maternal-fetal zinc status although data on human placental MT levels and influencing factors are scarce [29,10]. The possible physiological functions of metallothionein in the placenta include temporary zinc storage and regulation of the zinc flow to the fetus while restricting toxic metal transfer [29], and protection against the embryotoxic and teratogenic effects of zinc deficiency [23]. In our study, placental MT was not influenced by maternal zinc supplementation and did not correlate with maternal biochemical indices of zinc status, probably due to the small sample size. However, the values observed were, on average, 40% higher than those observed in a previous study in women with zinc dietary intakes of 13 mg/d [10], consistent with the proposed role of placenta MT in ensuring the zinc transfer to the fetus under less favorable conditions of maternal zinc status and dietary zinc intake.

CONCLUSIONS

Taken together, the results of this study indicate that measurement of RBCMT together with other biochemical zinc indices has value for understanding physiological adjustments in zinc homeostasis during pregnancy. However, RBCMT appears to not be responsive during pregnancy to changes in zinc status achieved with zinc supplements.

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REFERENCES

1. Sandstead HH. Zinc deficiency. A public health problem? Am J Dis Child 1991;145:853–859. [PubMed: 1858720]
2. Caulfield LE, Zavaleta N, Shankar AH, Merialdi M. Potential contribution of maternal zinc supplementation during pregnancy to maternal and child survival. Am J Clin Nutr 1998;68:499S–508S. [PubMed: 9701168]
3. Osendarp SJ, West CE, Black RE. Maternal Zinc Supplementation Study Group. The need for maternal zinc supplementation in developing countries: an unresolved issue. J Nutr 2003;133:817S–827S. [PubMed: 12612160]
4. International Zinc Nutrition Consultative Group (IZiNCG). Assessment of the risk of zinc deficiency in population and options for its control. Food and Nutrition Bulletin 2004;25:S91–S202.
5. Grider A, Bailey LB, Cousins RJ. Erythrocyte metallothionein as an index of zinc status in humans. Proc Natl Acad Sci USA 1990;87:1259–1262. [PubMed: 2304897]
6. Thomas EA, Bailey LB, Kauwell GA, Lee DY, Cousins RJ. Erythrocyte metallothionein response to dietary zinc in humans. J Nutr 1992;122:2408–2414. [PubMed: 1453226]
7. Sullivan VK, Burnett FR, Cousins RJ. Metallothionein expression is increased in monocytes and erythrocytes of young men during zinc supplementation. J Nutr 1998;128:707–713. [PubMed: 9521632]
8. Cao J, Cousins RJ. Metallothionein mRNA in monocytes and peripheral blood mononuclear cells and in cells from dried blood spots increases after zinc supplementation of men. J Nutr 2000;130:2180–2187. [PubMed: 10958810]
9. Zapata CL, Melo MR, Donangelo CM. Maternal, placental and cord zinc components in healthy women with different levels of serum zinc. Biol Neonate 1997;72:84–93. [PubMed: 9267674]
10. Zapata CL, Simoes TM, Donangelo CM. Erythrocyte metallothionein in relation to other biochemical zinc indices in pregnant and nonpregnant women. Biol Trace Elem Res 1997;57:115–124. [PubMed: 9282258]
11. Donangelo CM, Zapata CL, Woodhouse LR, Shames DM, Mukherjea R, King JC. Zinc absorption and kinetics during pregnancy and lactation in Brazilian women. Am J Clin Nutr 2005;82:118–124. [PubMed: 16002809]
12. Sacco LM, Caulfield LE, Zavaleta N, Retamozo L. Dietary pattern and usual nutrient intakes of Peruvian women during pregnancy. Eur J Clin Nutr 2003;57:1492–1497. [PubMed: 14576764]
13. Caulfield LE, Z N, Figueroa A, Leon Z. Adding zinc to prenatal iron and folate supplements does not affect duration of pregnancy or size at birth in Peru. J Nutr 1999a;129:1563–1568. [PubMed: 10419991]
14. Merialdi M, Caulfield LE, Zavaleta N, Figueroa A, Costigan KA, Dominici F, Dipietro JA. Randomized controlled trial of prenatal zinc supplementation and fetal bone growth. Am J Clin Nutr 2004a;79:826–830. [PubMed: 15113721]
15. Merialdi M, Caulfield LE, Zavaleta N, Figueroa A, Dominici F, Dipietro JA. Randomized controlled trial of prenatal zinc supplementation and the development of fetal heart rate. Am J Obstet Gynecol 2004b;190:1106–1112. [PubMed: 15118650]
16. Eaton DL, Toal BF. Evaluation of the Cd/Hemoglobin affinity assay for the rapid determination of metallothionein in biological tissues. Toxic Appl Pharmocol 1982;66:134–142.
17. Waalkes MP, Poisner AM, Wood GW, Klaassen CD. Metallothionein-like proteins in human placenta and fetal membranes. Toxicol Appl Pharmacol 1984;74:179–184. [PubMed: 6740669]
18. Fung EB, Ritchie LD, Woodhouse LR, Roehl R, King JC. Zinc absorption in women during pregnancy and lactation: a longitudinal study. Am J Clin Nutr 1997;66:80–88. [PubMed: 9209173]
19. Caulfield LE, Zavaleta N, Figueroa A. Adding zinc to prenatal iron and folate supplements improves maternal and neonatal zinc status in a Peruvian population. Am J Clin Nutr 1999b;69:1257–1263. [PubMed: 10357748]

20. Zavaleta N, Caulfield LE, Garcia T. Changes in iron status during pregnancy in Peruvian women receiving prenatal iron and folic acid supplements with or without zinc. Am J Clin Nutr 2000;71:956–961. [PubMed: 10731503]
21. King JC. Determinants of maternal zinc status during pregnancy. Am J Clin Nutr 2000;71:1334S–1343S. [PubMed: 10799411]
22. O'Brien KO, Zavaleta N, Caulfield LE, Wen J, Abrams SA. Prenatal iron supplements impair zinc absorption in pregnant Peruvian women. J Nutr 2000;130:2251–2255. [PubMed: 10958820]
23. Davis SR, Cousins RJ. Metallothionein expression in animals: a physiological perspective on function. J Nutr 2000;130:1085–1088. [PubMed: 10801901]
24. Cousins RJ, Lee-Ambrose LM. Nuclear zinc uptake and interactions and metallothionein gene expression are influenced by dietary zinc in rats. J Nutr 1992;122:56–64. [PubMed: 1370327]
25. Maret W. The function of zinc metallothionein: a link between cellular zinc and redox state. J Nutr 2000;130:1455S–1458S. [PubMed: 10801959]
26. Powell SR. The antioxidant properties of zinc. J Nutr 2000;130:1447S–1454S. [PubMed: 10801958]
27. Li TY, Kraker AJ, Shaw CF 3rd, Petering DH. Ligand substitution reactions of metallothioneins with EDTA and apo-carbonic anhydrase. Proc Natl Acad Sci U S A 1980;77:6334–6338. [PubMed: 6779278]
28. Yamashita K, Ohno H, Doi R, Mure K, Ishikawa M, Shimizu T, Arai K, Taniguchi N. Distribution of zinc and copper in maternal and cord blood at delivery. Biol Neonate 1985;48:362–365. [PubMed: 3936551]
29. Goyer RA, Haust MD, Cherian MG. Cellular localization of metallothionein in human term placenta. Placenta 1992;13:349–355. [PubMed: 1438083]
30. O'Brien KO, Zavaleta N, Abrams SA, Caulfield LE. Maternal iron status influences iron transfer to the fetus during the third stage of pregnancy. Am J Clin Nutr 2003;77(4):924–930. [PubMed: 12663293]

Table 1

Maternal characteristics of 158 Peruvian women with biochemical status information by supplement type.[1]

	Iron + folic acid + zinc	Iron + folic acid
N	72	86
Maternal age (y)	23.1 (4.5)	23.4 (4.9)
Maternal body mass index (kg/m^2)	23.0 (3.0)	23.7 (3.4)
Maternal height (cm)	152.6 (5.1)	152.1 (5.1)
Hemoglobin concentration at enrollment (g/L)	121 (9)	121 (10)
Parity (%)		
0	59.7	58.1
1	23.6	27.9
2	13.9	8.1
>2	2.8	5.8
Gestation at enrollment (wk)	12.9 (1.9)	12.9 (1.9)

Presented values are means (standard deviations) or percent.

[1] No statistical significant difference by supplement type ($p<0.05$)

Table 2

Characteristics of the 158 Peruvian newborns by supplement type.[1]

	Iron + folic acid + zinc	Iron + folic acid
N	72	86
Gestational age (wk)	39.7 (1.1)	39.8 (1.0)
Sex (%)		
Female	48.6	50.6
Male	51.4	49.4
Birth weight (g)	3239 (423)	3251 (378)
Birth length (cm)	49.7 (2.0)	49.8 (1.9)
Head circumference (cm)	34.6 (1.4)	34.7 (1.2)

Presented values are means (standard deviations) or percent

[1] No statistical significant difference by supplement type ($P<0.05$)

Table 3

Changes in indicators[a] during pregnancy by prenatal supplement type

	Iron + folic acid + zinc	Iron + folic acid
RBC MT (nmol/g protein)		
N	72	86
12 wk [b]	1.6 (0.5)	1.7 (0.5)
28 wk	1.7 (0.5)	1.8 (0.4)
36 wk	1.8 (0.6)	1.8 (0.5)
change (36 wk – 12 wk)	0.2 (0.5)	0.1 (0.6)
cord	1.8 (0.5)	1.9 (0.4)
Placental MT		
(N= 14; 16) (nmol/g)	0.65 (0.17)	0.78 (0.17)
Plasma zinc (umol/L)		
N	72	86
12 wk[b]	10.1 (2.1)	10.2 (2.0)
28 wk	8.7 (1.5)	8.4 (1.8)
36 wk	8.4 (1.7)	8.3 (1.6)
change (36 wk – 12 wk)	−1.7 (1.7)	−1.9 (1.8)
cord	13.6 (2.7)	13.5 (2.4)
Urinary zinc (sqrt of umol/L)		
N	72	85
12 wk[b,c]	2.0 (0.8)	2.2 (0.8)
28 wk	1.8 (0.7)	1.7 (0.7)
36 wk	1.8 (0.7)	1.7 (0.6)
change (36 wk – 12 wk)	−0.2 (1.0)	−0.5 (0.9)
RBC zinc (μg/g protein)		
N	14	15
12 wk	106.7 (24.3)	93.1 (21.3)
28 wk	116.7 (38.3)	112.2 (51.7)
36 wk	125.3 (25.4)	98.9 (36.3)
change (36 wk – 12 wk)	18.6 (25.9)	5.8 (26.9)
cord	37.8 (23.3)	30.9 (19.4)
Plasma albumin (mg/dL)		
N	39	47
12 wk[b]	4.2 (0.4)	4.3 (0.4)
28 wk	3.4 (0.3)	3.4 (0.3)
36 wk	3.2 (0.3)	3.1 (0.3)
change (36 wk – 12 wk)	−1.1 (0.3)	−1.2 (0.3)
Plasma ferritin (μg/L)		
N	(70,72,72,70)	(86,85,86,86)
12 wk[b]	30.1 (19.4)	29.3 (19.5)
28 wk	14.2 (12.8)	14.7 (12.8)
36 wk	16.7 (13.3)	17.3 (10.6)
change (36 wk – 12 wk)	−13.4 (16.1)	−12.0 (18.6)
cord	74.2 (19.8)	75.8 (19.1)
Hemoglobin (g/L)		
N	72	86
12 weeks [b]	121.4 (9.2)	121.3 (10.4)
28 weeks	115.1 (10.5)	115.1 (10.0)
36 weeks	119.8 (12.6)	121.5 (12.5)
change (36wk-12wk)	−1.7 (13.0)	0.3 (13.7)
Hematocrit (%)		
N	72	86
12 wk [b]	35.6 (2.7)	35.5 (3.2)
28 wk	33.9 (3.1)	34.3 (2.7)
36 wk	35.3 (3.6)	35.5 (3.5)
change (36 wk – 12 wk)	−0.3 (3.6)	0.1 (4.2)

[1] Presented values are means (standard deviations)

[a] Not different by type of prenatal supplement in a repeated measures ANOVA ($P > 0.05$)

[b] Different by time in a repeated measures ANOVA, $P < 0.05$

[c] Different by supplement over time (interaction) in a repeated measures ANOVA, $P < 0.05$

Table 4

Correlation matrices for biochemical indicators during pregnancy among 158 Peruvian mothers and newborns

	12 wk	28 wk	36 wk	Cord/Placenta
RBC MT				
12 wk	1.00	0.41*	0.37*	0.48*/−0.01
28 wk		1.00	0.48*	0.33*/0.10
36 wk			1.00	0.29*/0.09
cord/placenta				1.00/0.25
Plasma zinc				
12 wk	1.00	0.51*	0.55*	0.07
28 wk		1.00	0.53*	−0.04
36 wk			1.00	0.08
cord				1.00
Urinary zinc	Spearman's r			
12 wk		0.33*	0.19*	--
28 wk	1.00	1.00	0.38*	--
36 wk			1.00	--
RBC zinc				Spearman's r:
12 wk	1.00	0.43*	0.62*	0.58*
28 wk		1.00	0.60*	0.72*
36 wk			1.00	0.64*
cord				1.00
Plasma albumin				
12 wk	1.00	0.54*	0.55*	--
28 wk		1.00	0.67*	--
36 wk			1.00	--
Hemoglobin				
12 wk	1.00	0.34*	0.31*	--
28 wk		1.00	0.54*	--
36 wk			1.00	--
cord				--
Hematocrit				
12 wk	1.00	0.27*	0.27*	--
28 wk		1.00	0.52*	--
36 wk			1.00	--
cord				--
Plasma Ferritin	Spearman's r			
12 wk	1.00	0.40*	0.42*	−0.03
28 wk		1.00	0.65*	0.04
36 wk			1.00	0.04
cord				1.00

* Statistically different from zero, $P < 0.05$.

Table 5

Correlations of biochemical indicators with MT during pregnancy

	Maternal RBC MT			Cord RBC MT	Placenta MT
	12 wk	**36 wk**	**change (36 wk - 12 wk)**		
Plasma zinc					
12 wk	0.02	−0.18*	−0.18*	−0.12	0.12
36 wk	--	−0.01	−0.01	−0.10	−0.10
36 wk - 12wk	--	0.20*	0.20*	0.04	−0.30
Urinary zinc (Spearman's)					
12 wk	−0.02	−0.10	−0.10	0.01	0.09
36 wk	--	0.03	−0.07	0.01	−0.16
36 wk – 12 wk	--	0.12	0.08	−0.01	−0.11
RBC zinc					
12 wk	−0.14	0.42*	0.43*	0.03	−0.20
36 wk	--	0.29	0.29	0.05	0.01
36 wk – 12 wk	--	−0.01	−0.01	0.05	0.19
Plasma Albumin					
12 wk	−0.22*	−0.21#	0.04	−0.12	−0.16
36 wk	--	−0.04	0.001	−0.02	−0.17
36 wk - 12 wk	--	0.21#	−0.04	0.12	0.02
Hemoglobin					
12 wk	−0.02	−0.10	−0.07	0.10	−0.20
36 wk	--	−0.11	−0.13	−0.01	−0.37*
36 wk – 12 wk	--	−0.03	−0.07	−0.08	−0.26
Hematocrit					
12 wk	−0.04	−0.04	−0.001	0.08	−0.27
36 wk	--	−0.06	−0.13	−0.01	−0.35#
36 wk - 12 wk	--	−0.03	−0.12	−0.07	−0.15
Plasma Ferritin (Spearman's)					
12 wk	−0.19*	−0.18*	0.02	−0.17*	0.05
36 wk	--	−0.14	−0.05	−0.05	−0.17
36 wk – 12 wk	--	0.14	−0.02	0.14	−0.25

*P < 0.05

#P < 0.06