

## Original

# Dietary supplements for the lactating adolescent mother: influence on plasma micronutrients

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## Abstract

**Introduction:** The nutritional status of micronutrients in lactating adolescent women is crucial to guarantee an adequate secretion of these in breast milk and, consequently, an adequate nutritional status of children. Hence, more attention should be given to micronutrient status of adolescent mother who breastfeed. This study aimed to evaluate the influence of multimicronutrients supplementation upon nutritional status of iron, copper, zinc and calcium of lactating adolescent mother from low socioeconomic status in Rio de Janeiro/Brazil.

**Methods:** We conducted a randomized, placebo-controlled trial. During 60 days, 36 adolescents were allocated into two groups: supplemented group (SG) with 17 volunteers, receiving daily multimicronutrients supplement and the placebo group (PG) with 19 volunteers, receiving an inert compound. Plasma iron, copper, zinc and calcium and hemoglobin were determined at 7, 11 and 15 of the postpartum weeks (PPW). The effect of supplementation was analyzed by analysis of variance, comparing the differences between groups and within groups.

**Results:** The average age of volunteers was  $17.1 \pm 0.8$  for the supplemented group and  $16.3 \pm 1.4$  for the placebo group. We observed an increase in the mean concentration of zinc ( $p < 0.05$ ) and hemoglobin ( $p < 0.05$ ) in the SG during the study (60 days), while the PG showed reduction ( $p < 0.05$ ) in the mean concentration of copper between the 7<sup>th</sup> and 11<sup>th</sup> PPW.

**Conclusion:** The results of this study show that supplementation with multimicronutrients exerted positive effect on hemoglobin, copper and zinc.

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Key words: *Supplementation. Micronutrients. Lactation. Adolescents.*

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## SUPLEMENTOS DIETÉTICOS PARA LA MADRE ADOLESCENTE EN PERIODO DE LACTANCIA: SU INFLUENCIA EN MICRONUTRIENTES DEL PLASMA

## Resumen

**Introducción:** Debido a lo estado nutricional relativo a los micronutrientes de la nutriz ser de fundamental importancia para la adecuada secreción de leche materna, y en consecuencia un adecuado estado nutricional de los niños, se debe prestar una mayor atención a la situación de los micronutrientes de la madre lactante. El trabajo tuvo como objetivo estudiar la influencia de la suplementación con multimicronutrientes en lo estado nutricional de lo hierro, cobre, zinc y lo calcio de madres lactantes adolescentes de bajo nivel socioeconómico en Rio de Janeiro/Brasil.

**Métodos:** Se realizó un estudio con un ensayo clínico, aleatorizado, controlado con placebo. Durante 60 días, 36 jóvenes lactantes fueron estudiadas y distribuidas en dos grupos: el grupo suplementado (GS) con 17 voluntarias que recibieron un compuesto con multimicronutrientes y el grupo placebo (GP), con 19 voluntarias que recibieron un compuesto inerte. En todas las participantes del estudio se determinaron las concentraciones plasmáticas de hierro, cobre, calcio y zinc y de hemoglobina con 7, 11 y 15 semanas después del parto (SDP). El efecto de los suplementos se analizó por test de comparación de medias (Anova two way) entre los grupos y dentro de los grupos.

**Resultados:** La edad media de las voluntarias fue de  $17 \pm 0,8$  años para el GP y  $16,3 \pm 1,4$  para el GP. Se observó un aumento en las concentraciones medias de zinc y hemoglobina ( $p < 0,05$ ) en el GS durante el estudio (60 días) y la reducción ( $p < 0,05$ ) de cobre entre las 7<sup>a</sup> y 11<sup>a</sup> SDP en el GP.

**Conclusión:** Los resultados de este estudio muestran que la suplementación con multimicronutrientes tiene un efecto positivo en las concentraciones de hemoglobina, cobre y zinc.

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Palabras clave: *Suplementación. Micronutrientes. Lactancia. Adolescentes.*

## Abbreviations

BRP: Binding retinol protein.  
Ca: Calcium.  
Cu: Copper.  
Fe: Iron.  
ICP-OES: Inductively coupled plasma optical emission spectrometer.  
min: Minutes.  
PG: Placebo group.  
PPW: Postpartum week.  
RPM: Rotation per minute.  
SG: Supplemented group.  
UFF: Federal Fluminense University.  
UL: Tolerable upper intake levels.  
Zn: Zinc.

## Introduction

Gestation and lactation are anabolic processes controlled by hormones that distribute nutrients to highly specialized maternal tissues, such as placenta and mammary glands, and also transfer nutrients to fetal and neonatal development.<sup>1</sup> When gestation and lactation occur in adolescents, these requirements surpass the recommendations for this phase of life. Adolescents require nutrients for their own growth and physical development, which during the gestational-puerperal cycle is added to requirements for fetal growth and lactation. Hence, nutritional status of the adolescent mother is a crucial factor to be observed when it comes to prevention of maternal and newborn health risks.<sup>2</sup> Little is known about the impact of lactation upon maternal nutritional status of adolescent. Maternal health assistance is concentrated in gestation and in Brazil or in other countries there is scarce information about nutritional status of adolescents during lactation.

Lactation also represents a period of life cycle characterized by increased nutrients requirements, which can be acquired exclusively or partially by the diet.<sup>3</sup>

Micronutrients deficiency is still an important public health concern in Brazil. Regional researches at different parts of the country have been efficacious in identifying groups more prone to micronutrients deficiency and that such deficiencies are not restricted to less privileged regions of the country or even related with poverty or adverse economical conditions.<sup>4</sup>

Much as micronutrient deficiency can occur isolated, however it is usual to find combined deficiency. Thus, more attention should be drawn to micronutrients nutritional status in maternal-childish group, taking into account interactions that happen in mineral metabolism.<sup>4</sup>

It becomes easy to conceive the importance of carrying out studies aiming at getting better knowledge of the factors that can affect the nutritional status of adolescents. In this way, we aimed to evaluate the benefits

from multimicronutrients supplementation upon nutritional status of lactating adolescents from low income.

To gain a better understanding of these problems, a food supplementation trial was performed employing a specific nutritional balanced supplement. This supplement provides about 100% the recommended daily intake of trace minerals. The product administered during the study is commercially available in Brazil.

## Subjects and methods

### *Area studied*

The study was carried out with adolescent women living in Rio de Janeiro and Niterói and their surrounding area. Rio de Janeiro and Niterói are big towns, close to the sea, in the south-east of Brazil.

### *Study design and subjects*

The population under study comprised healthy adolescent mothers. The mothers had poor socioeconomic status. They were recruited at the maternity unit from Federal University of Rio de Janeiro in Rio de Janeiro and at Malu Sampaio policlinic of specialties of women health in Niterói. All of them were non-vegetarian, with normal weight gain during pregnancy and had uncomplicated pregnancies, labors and deliveries. None of the women experienced more than minor problems during pregnancy. This research project was approved by the Ethics committee in research from Federal Fluminense University (UFF).

The characteristics and aim of the study were explained in detail and all subjects interested gave informed consent. A total of 36 adolescent women were enrolled in the study. The subjects intended to breastfeed exclusively for at least 16 weeks.

The breastfeeding adolescent mothers were divided into two groups: Supplemented Group (SG) with lactating adolescent mothers given a multivitamin and multimineral supplement to their traditional diet (n = 17); and Placebo Group (PG) with lactating adolescent eating a typical Brazilian diet without supplements (n = 19). Sample randomization of volunteers in the groups were in blocks, which is characterized by the formation of blocks with a fixed number of individuals, equal size, in which the treatments proposed are distributed and followed until the end of the experiment. Women in supplemented group were instructed to take a daily dietary without further dietary modifications. The nutritional supplement provided 18 mg of Iron (ferrous fumarate), 15 mg of zinc (zinc oxide), 2 mg of copper (cupric oxide) and 162 mg of calcium (calcium phosphate dibasic) and others minerals and vitamins. The composition of the supplement did not surpass tolerable upper intake levels (UL) according to Institute of Medicine<sup>5</sup> to lactating adolescents.

### Sample collections and analyses

Blood samples were collected at 7, 11 and 15 weeks of postpartum and were obtained in both groups. Morning fasting blood samples (10 mL) were obtained by venous puncture and transferred into tubes containing heparin as an anticoagulant. Disposable materials free of trace minerals were used during sample collection and processing. Aliquots of whole blood were used for determination of hematocrit and hemoglobin concentrations. Blood samples were centrifuged at 3500 rpm for 15 min for separation of plasma.

Hematocrit was determined by a conventional capillary technique. Blood hemoglobin was measured by the cyanomethemoglobin assay (Bioclin Kit, Belo Horizonte, Brazil).

All samples of plasma were analyzed for mineral content of zinc, copper, iron and calcium. Plasma was mineralized in a high-pressure closed container with microwave heating (MarsXpress-Cem Corporation). Assay of microelements was carried out in an inductively coupled plasma optical emission spectrometer (ICP OES) (Jobin Yvon-Ultima 2). Individual storage solutions containing 1,000 mg L<sup>-1</sup> of Fe, Cu, Zn and Ca were used, after adequate dilution, in order to prepare analytic curves in the following concentrations: 0.1; 0.5; 1.0 and 2.5 mg L<sup>-1</sup>.

### Anthropometric study

Anthropometric data was collect in the morning. Weight was recorded on a stationary balance (Filizola®), accurate to within 100 g. Stature was evaluated, measuring the distance from vertex to the ground using a stadiometer, with barefooted adolescents in orthostatic position, with feet together and in respiratory apnea.

### Statistical analysis

Statistical analyses of data were carried out comparing means with a general analyses of variance (ANOVA).

**Table I**  
Characteristics of the adolescente women in the groups studied with 7 postpartum weeks

Characteristic	Supplemented group	Placebo group
No. of subjects	17	19
Age (y)	16.3 ± 1.4	17.1 ± 0.8
Postmenarcheal period (y)	4.4 ± 1.8	4.5 ± 1.8
Body mass index (kg/m <sup>2</sup> )	23 ± 3.8	22.1 ± 5.9
Hematocrit (%)	38.5 ± 4.0	39.3 ± 1.9
Blood hemoglobin (g/dL)	12.1 ± 1.2	12.9 ± 1.4
Plasma iron (mmol/L)	9.9 ± 4.1	10.1 ± 4.9
Plasma zinc (µmol/L)	16.4 ± 8.2	11.7 ± 7.7
Plasma copper (µmol/L)	15.4 ± 3.8 <sup>a</sup>	19.7 ± 6.2 <sup>b</sup>
Plasma calcium (mmol/L)	2.2 ± 0.4	2.1 ± 0.2

Values are presented as mean ± SD. Different superscript letters in the same row denote a significant difference between groups ( $p < 0.05$ ).

This studied the effect of times (postpartum weeks 7, 11 e 15), groups (supplemented group and placebo group) and interactions, i.e., differences between groups in regard to the changes of means at the studied times. Within each group, a *t*-test was used, taking 7<sup>th</sup> postpartum week as the control value after verification by ANOVA that means of 7<sup>th</sup> postpartum week did not differ significantly. Differences were considered significant if  $p < 0.05$ .

### Results

Descriptive characteristics of the lactating adolescent women studied at 7 postpartum weeks (PPW) (baseline) are presented in table I. The two groups of adolescents were similar in all characteristics, except for plasma copper. Levels of plasma minerals for lactating adolescent mothers during the study are reported in table II.

**Table II**  
General biochemical characteristics of lactating adolescents throughout the experiment. At the 7<sup>th</sup> postpartum week, 11<sup>th</sup> postpartum week and 15<sup>th</sup> postpartum week

Indicators	7 Postpartum weeks			11 Postpartum weeks			15 Postpartum weeks		
	Supplemented (n = 17)	Placebo (n = 19)	<i>P</i>	Supplemented (n = 12)	Placebo (n = 10)	<i>P</i>	Supplemented (n = 10)	Placebo (n = 10)	<i>P</i>
Hemoglobin (g/dL)	12.9 ± 1.4 <sup>a</sup>	12.1 ± 1.2	<i>ns</i>	14.6 ± 1.4 <sup>b</sup>	11.2 ± 2.9	0.0018	15.1 ± 2.5 <sup>b</sup>	12.4 ± 1.3	0.0072
Hematocrit (%)	39.3 ± 1.9	38.5 ± 4.0	<i>ns</i>	39.2 ± 1.8	39.8 ± 1.1	<i>ns</i>	40.1 ± 2.2	38.3 ± 4.2	<i>ns</i>
Plasma Iron (mmol/L)	9.9 ± 4.1	10.1 ± 4.9	<i>ns</i>	9.8 ± 4.5	10.3 ± 5.0	<i>ns</i>	10.8 ± 5.0	12.2 ± 4.1	<i>ns</i>
Plasma Copper (µmol/L)	15.4 ± 3.8	19.7 ± 6.2 <sup>a</sup>	0.01	15.6 ± 4.3	14.9 ± 0.7 <sup>b</sup>	<i>ns</i>	15.2 ± 2.8	15.1 ± 5.3 <sup>a</sup>	<i>ns</i>
Plasma Zinc (µmol/L)	16.4 ± 8.2 <sup>a</sup>	11.7 ± 7.7	<i>ns</i>	26.9 ± 15.8 <sup>a</sup>	13.1 ± 5.3	0.016	30.6 ± 8.1 <sup>b</sup>	10.3 ± 2.3	< 0.0001
Plasma calcium (mmol/L)	2.2 ± 0.4	2.1 ± 0.2	<i>ns</i>	2.1 ± 0.2	2.4 ± 0.2	0.0022	2.3 ± 0.4	2.3 ± 0.1	<i>ns</i>

Values are presented as mean ± SD. Different superscript letters in the same row denote a significant difference between postpartum weeks (analysis of variance,  $P < 0.05$ ).

### *Indicators of iron nutritional status*

Although hemoglobin levels were adequate at 7<sup>th</sup> PPW, around 20% of supplemented group and 37% of placebo group volunteers had anemia based on cutoff values established for hemoglobin (12 g/dL).<sup>6</sup> Concerning hematocrit, 36.8% of adolescents from placebo group presented values under the normal range (36%).<sup>6</sup> However, none of the volunteers from the supplemented group presented abnormal values. At 11<sup>th</sup> PPW, mean concentration of hemoglobin was above the reference values in the supplemented group. Conversely, placebo group showed mean concentration below to the normal value and significantly smaller than the supplemented group ( $p = 0.0018$ ). As for hematocrit, mean values were within the normal range for both groups. Differently from observations at 7<sup>th</sup> PPW, none of the volunteers from supplemented group were anemic at 11<sup>th</sup> PPW. In contrast, placebo group showed 20% of participants with anemia, without alterations in hematocrit. Evaluating these parameters at 15<sup>th</sup> PPW, means of hemoglobin and hematocrit were within the normal range for both groups, but the average of hemoglobin was higher in the supplemented group ( $p = 0.0072$ ).

Mean concentrations of plasma iron, at 7<sup>th</sup> PPW, were according to the normal values (7.2 mmol/L),<sup>7</sup> being not different between the groups. However, frequencies of inadequacy were 35.3% and 21% for supplemented and placebo groups, respectively. At 11<sup>th</sup> PPW mean concentrations remained similar between the groups and above the reference value. It was observed reduction in the frequency of inadequacy in the supplemented group, which showed 16.7% of volunteers with values lower than the ideal (7.2 mmol/L)<sup>7</sup>, whereas in placebo group this frequency increased to 30%. At 15<sup>th</sup> PPW mean from both groups were above the suitable value, with frequency of inadequacy of 20% in the supplemented group and 20% in the placebo group. As the study went by, means for both groups did not suffer modifications, maintaining the concentrations found at 7<sup>th</sup> PPW.

### *Plasma minerals*

At 7<sup>th</sup> PPW, mean concentrations of plasma copper, for both groups, were above normal values ( $> 10 \mu\text{mol/L}$ ).<sup>5</sup> On the other hand, placebo group presented higher concentration ( $p = 0.01$ ) when compared to the supplemented group. At this moment of the study, none of the volunteers presented inadequate concentration in the supplemented group, whereas 5.9% of placebo group showed concentration below to  $10 \mu\text{mol/L}$ . At 11<sup>th</sup> PPW, means remained above the cutoff value and the difference observed at 7<sup>th</sup> PPW disappeared and none of the volunteers from both groups presented copper concentration below to reference value. At 15<sup>th</sup> PPW, means were kept above the normal value, without differences between the groups. However, placebo

group almost doubled the frequency of copper inadequacy (10%) when compared to 7<sup>th</sup> PPW, emphasizing that supplemented group remained without volunteers under the normal value. After means throughout the experiment were analyzed, we perceived that placebo group presented diminishing in its values between the 7<sup>th</sup> PPW and the 15<sup>th</sup> PPW, whereas supplemented group did not show modifications during the study.

At baseline, 7<sup>th</sup> PPW, mean concentrations of zinc from both groups were above the cutoff value ( $9.2 \mu\text{mol/L}$ ),<sup>8</sup> being not different between them. Frequency of inadequacy observed during this week was 41.2% for the supplemented group and 47.4% for placebo group. At 11<sup>th</sup> PPW, means remained above the cutoff value, with the supplemented group showing values superior to placebo group ( $p = 0.016$ ). It was not found volunteer with low concentrations of zinc in the supplemented group. Nevertheless, in placebo group a frequency of inadequacy of 30% was observed. At 15<sup>th</sup> PPW means from groups remained adequate and the supplemented group maintained superior concentration in comparison with placebo group ( $p < 0.0001$ ). In this week, only placebo group presented volunteers (40%) with plasma concentration of zinc below to cutoff values. Supplemented group had increase in the concentration of zinc ( $p = 0.022$ ) during the experiment, which was not found in placebo group.

At 7<sup>th</sup> PPW, the mean concentration of plasma calcium, in both groups, were adequate, within the normal range (between 2.2 and 2.5 mmol/L),<sup>9</sup> being 58.8% of volunteers of supplemented group with values below  $2.2 \text{ mmol/L}$  and 57.9% of placebo group with values below to this. Means were not different in this week. Plasma mean concentration of supplemented group at 11<sup>th</sup> PPW was below the normal value ( $p = 0.0022$ ) and under the mean from placebo group. Consequently, supplemented group presented frequency of volunteers with inadequate concentrations (72.7%) superior to placebo group (20%). At 15<sup>th</sup> PPW, means are adequate and did not differ between the groups. Only the supplemented group showed 40% of adolescents with calcium values below the cutoff. It was not verified modifications in calcium concentrations throughout the experiment.

### **Discussion**

Mean levels of hematocrit were adequate in the two groups and did not differ between them at the 3 specific postpartum weeks that were studied. Our results resembled values found by Azeredo & Trugo<sup>10</sup> (38.5%) and Maia<sup>11</sup> (38%) in lactating adolescents from Rio de Janeiro and Meneses & Trugo<sup>12</sup> (38.4%) with lactating adults.

With relation to the effect of the supplementation of 18mg/day of iron upon the mean concentration of hematocrit, it was observed that both groups did not differ at the 3 specific evaluated postpartum weeks. These results show that in the first weeks after deliver-

ing the concentration of hematocrit seems to be influenced by gestational period. This can be explained by the fact that placebo group, which began the study with frequency of inadequacy of this indicator around 37%, finished the trial without inadequacy of this indicator of iron status. It can be suggested that a physiological recuperation happened once gestation implies an elevation of blood volume with resulting hemodilution of hematologic parameters.<sup>13</sup>

In the initial characterization of volunteers, around 7<sup>th</sup> PPW, the mean concentration of hemoglobin was above the normal in both groups. Similar values were reported by other researchers in the same period in lactating adolescents from Rio de Janeiro (11.9 g/dL<sup>10</sup> and 12 g/dL<sup>11</sup>) and with lactating adults (12.2 g/dL<sup>12</sup> and 13.4 g/dL<sup>14</sup>). At baseline, approximately 20% of supplemented group and 37% of placebo group had anemia. Azeredo et al.<sup>2</sup> studying lactating adolescents at the same period, found frequency of anemic volunteers (35%) similar to the observed in placebo group and superior to supplemented group.

As far as impact of iron supplement (18 mg/day) upon hemoglobin levels is concerned, it provoked significant rise in mean concentrations of hemoglobin at 15<sup>th</sup> PPW ( $p < 0.05$ ) in the supplemented group, which was not observed in placebo group. Likewise, Bruner et al.<sup>15</sup> reported similar results after supplementing 60 mg of elemental iron daily to non anemic and non lactating adolescents during 8 weeks. Supplemented girls showed higher values of hemoglobin, corroborating with present findings as placebo group presented increased frequency of anemic volunteers in the follow-up. Madhavan Nair et al.<sup>16</sup> observed that daily intake during 100 days of 12 mg of elemental iron in Indians women, average age of 22 years old, increased significantly hemoglobin levels. However, Gropper et al.<sup>17</sup> who studied the supplementation of 50 mg of elemental iron in non lactating women, average age of 27 years old from Alabama, USA, did not found differences concerning plasma iron, hemoglobin and hematocrit before and after the trial. Hence, present findings show that supplementation of 18 mg of elemental iron is able to maintain serum concentration of hemoglobin within adequate values, avoiding anemia during this biological period when maternal requirements increase due to the addition of newborn's requirements. It can be concluded that with only 18 mg/day of supplemented iron we observed the same effect of 60 mg of Elemental iron upon hemoglobin levels rising.

Our findings reveal that mean plasma concentrations of iron were suitable in both groups during the whole experiment. Mean concentration of plasma iron was close to values found by Azeredo et al.<sup>2</sup> (9.8  $\mu\text{mol/L}$ ) and Al-Awadi & Srikumar<sup>18</sup> (11.6  $\mu\text{mol/L}$ ) in nursing adolescents and adults, respectively.

Although supplemented group has shown rise in hemoglobin concentrations, plasma iron concentration remained unaltered. This occurred owing to the fact that the amount of iron in the supplement (18 mg) was

sufficient only to provide erythrocytes synthesis by bone marrow, but not to keep augmented levels of iron.

Significant clinical copper deficiency is rare in humans, suggesting that usual dietary intake provides the daily recommended amount suitably.<sup>19,20</sup> During lactation, child relies on maternal copper supply to reach adequate development.<sup>19</sup> Maia et al.<sup>11</sup> and Azeredo et al.<sup>2</sup> found mean copper values similar to our findings in nursing adolescents from Rio de Janeiro (18.7  $\mu\text{mol/L}$  e 19.7  $\mu\text{mol/L}$ , respectively) and also similar to Al-Awadi & Srikumar,<sup>18</sup> who found mean copper concentrations of 19.3  $\mu\text{mol/L}$  in nursing adults from Kuwait. Although mean plasma copper concentrations were within normal range at the beginning of the study for both groups, placebo group presented nearly 6% of with copper concentration below the normal, similar to the frequency reported by Azeredo et al.<sup>2</sup> in a study about nursing adolescents from Rio de Janeiro (8%).

At baseline, mean copper concentration of placebo group was higher than supplemented group, but this difference disappeared over the postpartum weeks. This elevated copper concentration, at the 7<sup>th</sup> PPW, can be explained by estrogen influence, a hormone found in high concentrations during gestation. Our volunteers might be under this effect due to the recent gestation period. Bedwal and Bahunga<sup>21</sup> reported that estrogen alters subcellular distribution of copper in liver and increases plasma copper levels through the induction of ceruloplasmin synthesis.

As for supplementation, Bügel et al.<sup>20</sup> observed that the supplementation with 3 mg of copper per Day during 28 days increased significantly serum copper concentration in studying non-lactating young women, between 21 and 28 years old from Denmark. In our study, supplementation of 2 mg of copper was capable of maintaining normal serum concentrations and avoiding inadequacy of this micronutrient in 100% of nursing adolescents at 11<sup>th</sup> and 15<sup>th</sup> PPW. On the other hand, in placebo group, there was a frequency of 10% of inadequacy for this mineral.

Zinc physiological role during rapid growth and development period emphasizes its importance during gestation and lactation periods. At postnatal period, zinc deficiency can affect growth and immune system development due to interaction with vitamin A, making the child more vulnerable, increasing morbimortality, given that zinc is required to hepatic synthesis and secretion of BRP (binding retinol protein), a protein responsible for vitamin A transport.<sup>4</sup>

Plasma zinc concentration is still considered an indicator to evaluate zinc status in populations, even though it represents only 0.1% of total body zinc.<sup>22</sup> The present study verified that nursing adolescents presented mean plasma zinc levels according to recommended values in the literature for this age and physiological period. Mean concentrations of placebo group was similar to other experiments with nursing adolescents (11.8  $\mu\text{mol/L}$ ),<sup>2,11</sup> whereas supplemented group presented values superior to these studies.

At the beginning of the study mean zinc concentrations of supplemented and placebo groups were equal, but over the postpartum weeks a difference between them was observed. Supplemented group presented higher concentration than placebo group and this can be accounted for supplement (15 mg) effect. According to Brown et al.,<sup>23</sup> changes in zinc concentration can be used as a practical indicator that zinc supplement was given, consumed and absorbed successfully by population exposed to the intervention. This finding corroborates with a study of Clark et al.,<sup>24</sup> who supplemented non-lactating adolescents from Sheffield, United Kingdom, with 15 mg/day of zinc, the same amount of our study, and observed significant rise of 28.6% in mean zinc concentration after 6 weeks. Placebo group showed an elevation of only 0.5% in the English trial, whereas in our study placebo group presented progressive decrease of zinc concentration, but without statistical significance besides a rise of 60% in mean zinc concentrations of the group supplemented with 15mg/day of zinc. It can be suggested that if our trial continued for 2 more months, it may be observed reduction of mean zinc concentrations in placebo group until inadequate values, indicating the positive effect of daily 15 mg zinc supplementation.

Other studies had results different from ours. Lopez de Romana et al.<sup>25</sup> supplemented healthy non-lactating women between 35 and 40 years old from Chile with 20 mg/day of zinc during 2 months and did not observe effects of the supplement upon zinc concentrations. Moreover, Osendarp et al.<sup>8</sup> studied the supplementation of 30 mg of zinc during 5 months in pregnant women and did not report any effect on plasma zinc concentration. Likewise, Donangelo et al.<sup>26</sup> provided 22 mg of zinc during 2.3 months and also did not observe positive effect of supplementation upon plasma zinc levels. Conflicting results observed are probably due to plasma zinc is homeostatically regulated<sup>25</sup> and each one of these studies was conducted at different stages of the reproductive cycle, where estrogen and prolactin can influence.

Lactation is a period of elevated demand of calcium owing to milk production. Physiological adaptations occur in the body during this period, ensuring an adequate transfer of calcium to mammary gland.<sup>27</sup> Those physiological adaptations include temporary loss of bone mass, elevation of calcium absorption and reduction of urinary excretion of calcium.<sup>28,29</sup> Low calcium intake can affect calcium homeostasis in lactation, increasing the efficiency of calcium absorption and renal conservation of this mineral.<sup>27</sup> Adolescence is a crucial period for bone mass acquirement and also of high demand of calcium, which can affect physiological adjustments during lactation. Total plasma calcium values in the present study, at 7<sup>th</sup> PPW, were within the normal range only in the supplemented group, whereas in the placebo group mean was bellow, but not statistically different from supplemented group. These values were smaller than values referred by the literature for

nursing adolescents (2.5 mmol/L<sup>2</sup>) and resembled values from nursing adults between 7<sup>th</sup> and 8<sup>th</sup> PPW with low calcium intake (2.0 mmol/L<sup>29</sup>). Throughout the study mean concentrations of calcium did not differ from values cited in the literature. At 11<sup>th</sup> PPW, placebo group presented a significantly higher mean than supplemented group and, therefore, smaller frequency of girls with abnormal plasma calcium concentration. However, this difference between the means was not found at 15<sup>th</sup> PPW, where means were within the normal range. As our study did not aim to supplement calcium because the amount of calcium in the supplement was only 162 mg (12.4% of the daily recommended intake), it was not observed effect of the supplementation upon plasma calcium levels once values from both groups were similar at the end of the trial. However, it was perceived over the weeks studied that supplemented group presented an elevated frequency of volunteers with plasma calcium concentrations below the normal value and this can stem from interaction among nutrients (iron, zinc and copper). These divalent metal ions may compete for the same protein (dmt1) to be transported in enterocyte, indicating the need for increasing calcium concentration in the supplement or even to supplement calcium apart from other minerals, at different times.

## Conclusion

Multimicronutrients supplementation was effective for the complete recuperation of anemia and maintenance of adequate concentrations of zinc in 100% of lactating adolescents studied. In relation to plasma copper, supplementation was sufficient to prevent from deficiency in 100% of the group at the end of the trail. Nevertheless, mean concentrations were not different. It should be highlighted the fact that a negative influence of the supplementation upon plasma calcium was observed owing to interactions among nutrients and the supplement.

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