



ORIGINAL ARTICLE

Iron, copper and zinc in adolescents during pubertal growth spurt

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Abstract

Objective: to examine iron, copper and zinc nutritional status and their correlation with Body Mass Index (BMI), serum and dietetic levels in adolescents during the pubertal growth spurt.

Methods: a descriptive cross-sectional study involving a sample of 47 adolescents out of 360 patients (19 boys, whose ages ranged from 12.3 to 16 years and 28 girls, whose ages ranged from 11.1 to 13.6 years), who were seen at a clinic for adolescents from March to December 1999. The variables analyzed were: Diet (24 hours Dietary Recall, Food Frequency Intake Questionnaire and Food Register Methods) to determine iron, copper and zinc intake; anthropometry (weight and height) to check BMI; biochemistry (measure of serum iron level through a *Diagnóstica* kit in vitro; ferritin through *Immulite* kit, and atomic absorption spectrophotometry for biochemical evaluation of serum iron, ferritin, copper and zinc. Spearman coefficient correlation was used for statistical analysis.

Results: forty seven adolescents during pubertal growth spurt showed adequate ingestion: iron (95% and 36%), copper (53% and 57%) and zinc (21% and 21%) in males and females, respectively. Most of them were eutrophic according to the BMI percentiles. Biochemically, boys presented normal values for serum iron and zinc in the whole sample, 95% for copper and 84% for ferritin. Girls also presented normal values for iron and zinc values in the whole sample, 96.4% for copper and 96% for ferritin. There were no statistically significant correlation between BMI and serum Fe, ferritin, Cu and Zn concentrations and between serum concentration and dietetic ingestion of the studied minerals, neither between serum iron and ferritin.

Conclusions: it is not clear if serum levels of Zn and Cu are floating during the growth process or if each adolescent has a stable level of these minerals during the pubertal growth spurt. Normal Fe, Cu and Zn serum levels in most adolescents evaluated may reflect the organism ability to accomplish homeostatic adjustments.

J Pediatr (Rio J) 2002; 78 (4): 327-34: iron, copper, zinc, adolescent, pubertal growth spurt.

Introduction

The process of sexual maturation and higher growth rate in adolescence usually start and finish in the second decade of life. Developmental age, as assessed by the stage of

sexual maturation and/or bone age, is more accurate for the study of adolescents than chronological age.¹ Given the normal variability of sexual maturation during puberty, same-age adolescents can be impubescent, pubescent, or present adult phenotypes.

The maximum rate of muscle growth occurs during the peak growth spurt in boys and after the growth spurt, along with menarche, in girls. Maturation and growth stages are involved in the nutritional diagnosis and prognosis and determine the nutritional requirements for each phase of

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Financially supported by: CAPES.

Manuscript received Dec 20 2001. Accepted for publication May 08 2002.

adolescence.² Therefore, appropriate nutrition is a basic health requirement for adolescents to properly express their genetic potential in terms of growth and development.³

Due to enhanced growth during adolescence, the requirement of some minerals is of paramount importance. An inappropriate diet during adolescence can substantially delay sexual maturation and growth and the excessive consumption of some foods can increase the risk for chronic diseases in adolescence.³ Zinc, copper and iron are essential trace elements involved in adolescent growth.⁴

Iron is essential for the expansion of blood volume and muscle mass. It has enzyme or metabolic functions, in addition to storing iron in the form of ferritin and hemosiderin in order to maintain homeostasis. When dietary iron is inadequate, iron stores are mobilized to maintain the production of hemoglobin and other iron-containing components.⁵ During puberty, serum ferritin levels increase in boys, while they stay stable in girls, both in adolescence and adult life, and will only reach the same levels presented by boys after menopause. Attention deficits, poor performance in intelligence tests, behavioral and mood changes, tiredness, and below-average school performance are associated with iron deficiency in adolescents.⁶ Young individuals, just like infants, contain much more copper per body weight than adults. Copper is necessary for growth, is an important factor for several enzyme systems, and is involved in the synthesis of hemoglobin, oxidizing ferrous iron into ferric iron, and in the formation of transferrin by means of ceruloplasmin. Zinc, which is an essential cofactor for nearly 200 enzymes, participates in cellular growth as a cofactor for enzymes necessary for the synthesis of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), and controls, among other functions, gonadal growth and development. Adolescents need more zinc/kg than adults, due to the role of this metal in sexual maturation and growth.⁷ According to what has been mentioned, it is important to assess the levels of iron, copper and zinc in adolescents during the pubertal spurt.

Methods

Cross-section study carried out in 1999 between March 1st and December 10, at a clinic of the Center for Nutrition, Feeding and Infant Development (NUNADI - Núcleo de Nutrição, Alimentação e Desenvolvimento Infantil), in the city of São Paulo, which attends to socioeconomically underprivileged, adolescents. A sample of 47 adolescents at pubertal spurt, of 360 who were usually attended to at this center, was chosen. Of the selected adolescents, 19 were males with a median age of 13.9 years (range between 12.3 and 16 years), 13 at stage G3, and six at stage G4; and 28 were females, with median age of 12.2 years (range between 11.1 and 13.6 years), two at M2 and 26 at M3.⁸ Adolescents with clinically evident acute or chronic diseases at consultation, and those who were using nutritional

supplements were not included in the study. Only those who were at pubertal spurt were included. Of a total of 50 patients at pubertal spurt, three patients were not included, since they did not turn up for exams; therefore, the final sample consisted of 47 adolescents. Clinical examination and assessment of pubertal stage were performed a qualified adolescent's physician, who also participated in this study. The other data were collected by the nutritionist, author of the present article.

The nutritional assessment consisted of the following methods: 24-hour diet recall, dietary frequency, and dietary record⁹ on four consecutive days, including one weekend day. The validated and standardized Virtual Nutri - USP¹⁰ program was used to calculate the intake of iron, copper and zinc. The calculated dietary intake was compared with the Recommended Dietary Allowances (RDA).¹¹

A platform scale (Filizola), graded every 100 grams, was used to check body weight. Before each of the adolescents stepped onto the scale, it was tared. The stadiometer of the scale was used to check height. Afterwards, the body mass index - BMI (weight/height²)¹² was calculated. The obtained values were compared with the reference values proposed by Must,¹³ and classified into thin, eutrophic, overweight and obese, according to the criteria established by Himes & Dietz.¹⁴

Biochemical assessment: fasting blood samples (eight to nine hours) were collected. The samples were stored at -18°C up to the moment of dosage. The serum was diluted with deionized water by means of the Milli-Q-Plus system (Millipore Co.), at the ratio of 1:5, and the concentrations of copper and zinc were determined by atomic absorption spectrophotometry (Perkin-Elmer, model 5.100), as follows: wavelength of 324.8nm (Cu) and 213.9nm (Zn); slit 0.7nm; acetylene/air oxidizing flame; energy 71 (Cu) and 63 (Zn); duplicate reading with an integration time of two seconds.

Standard solutions of 100mg metal/l (Perkin Elmer, PEN 4300183 for Cu and PEN 9300178 for Zn) were diluted in glycerol at 10% (Cu) and glycerol at 5% (Zn), resulting in standard working solutions of containing 0.1; 0.2; 0.3; 0.5; 1.0 and 2.0mg of Cu or Zn/l. The accuracy was monitored by the comparison with commercially available *Quality Control Standard 21 solution* (Perkin Elmer, PEN 9300281) for both elements. All the reagents used were analytical, and the flasks used were washed with acid.

To determine serum iron, the Diagnóstica CAT 015 in vitro kit, which adopts the modified Goodwin method (Ferrozine); and the Ferritin-Immulite kit, which uses an immunometric assay, was used for ferritin. The results were expressed in mg of element/dL, and the adopted reference values were 45 to 50mg/dL for iron, 70 to 140mg/dL for copper, 50 to 120mg/dl¹⁵ and above 12mg/l for ferritin,¹⁶ for both sexes.

In the statistical analysis, Spearman correlation coefficient¹⁷ was used to compare BMI and the serum

levels of iron, copper and zinc, and the intake of these trace elements with their respective serum concentrations. An alpha level of $\leq 5\%$ was adopted.

The study was approved by the Ethics and Research Committees of Universidade Federal de São Paulo and NUNADI, Health Secretariat of the State of São Paulo. A written informed consent was obtained from parents or guardians.

Results

With regard to the intake of micronutrients, iron, copper and zinc contents were appropriate in 18, 10 and 4 male adolescents, respectively. Among female adolescents, the intake of iron, copper and zinc was adequate in 10, 16 and 6 individuals, respectively (Table 1). Table 2 shows the mean daily intake of Fe, Cu and Zn in comparison with RDA. We observed that the mean intake of iron in male adolescents was above the RDA, while the mean intake of zinc is below the recommendation for both sexes.

Table 1 - Distribution of adolescents according to gender and iron, copper and zinc intake adequacy (inadequate, adequate)

Intake	Iron		Cooper		Zinc	
	Male n	Female n	Male n	Female n	Male n	Female n
Inadequate	1	18	9	12	15	22
Adequate	18	10	10	16	4	6
Total	19	28	19	28	19	28

The mean body weight and height of male adolescents were 47.5 ± 6.8 kg and 160.2 ± 7.7 cm, respectively, and the median BMI was $18\text{kg}/\text{m}^2$. Female adolescents presented a mean body weight of 43.4 ± 12.5 kg, mean height of 150.6 ± 8.3 cm, and a BMI of $17.5\text{kg}/\text{m}^2$. Table 3 shows that most part of the sample (16 males and 21 females) were eutrophic; two male adolescents were thin and one was overweight. Among females, two were thin, two were overweight and three were obese.

All adolescents had normal serum iron, with means of $104.31\text{mg}/\text{dL}$ and $96.99\text{mg}/\text{dL}$ for males and females, respectively. Ferritin level was low in three males and in one female adolescent, with a mean of 37.36 for males and of $33.28\text{mg}/\text{dL}$ for females. The serum levels of copper were normal in 18 male and 27 female adolescents (Tables 4 and 5). Table 4 shows a mean serum copper level of $98.82\text{mg}/\text{dL}$ in males and of $101.48\text{mg}/\text{dL}$ in females. Serum zinc concentration in male adolescents was $88.66\text{mg}/\text{dL}$, and $77.50\text{mg}/\text{dL}$ in female adolescents; therefore, all adolescents presented normal serum levels of zinc.

No correlation was found between BMI and the serum levels of iron, ferritin, copper and zinc in both sexes (Table 6). The serum levels of iron and copper did not show any correlation with the dietary intake of these minerals, both in males ($r = -0.139$ and 0.290) and females ($r = 0.094$ and -0.76). The adolescents showed a weak and negative correlation ($r = -0.48$) between the serum concentration and the dietary intake of zinc, with no statistical significance. No correlation was observed among female adolescents ($r = 0.336$). When ferritin and serum iron levels were considered, no correlation was found for both males ($r = 0.178$) and females ($r = 0.006$).

Table 2 - Mean daily intake of iron, copper and zinc of adolescents of both genders during the pubertal growth spurt

	Male n=19	Female n=28	RDA	
			Male	Female
Iron (mg)	16.41 ± 2.80 (10.99–20.93)	13.86 ± 3.74 (6.98–23.19)	12	15
Cooper (mg)	1.71 ± 0.63 (1.00–3.65)	1.77 ± 0.89 (0.71–5.15)	1.5 to 2.5	1.5 to 2.5
Zinc (mg)	11.93 ± 3.43 (9.25–19.10)	9.79 ± 3.38 (5.07–18.64)	15	12

Table 3 - Distribution of adolescents of both genders in the pubertal growth spurt according to the nutritional status calculated through the percentile of the body mass index – BMI

Adolescents	Slim ≤ p5	Eutrophic p5— p85	Overweight ≥ p85— p95	Obese ≥ p95
Male	2 (15.6–16.0)	16 (16.6–20.8)	1 (22.2)	0 (0)
Female	2 (14.0–14.3)	21 (15.1–20.8)	2 (22.2–25.8)	3 (26.3–33.3)

() variation of BMI values

Discussion

Even though the mean daily intake of copper in both males and females was according to the RDA, nine male adolescents and 12 female adolescents showed an intake below the minimum recommended level. With regard to zinc, most adolescents (15 males and 22 females) had an appropriate intake (Tables 1 and 2). This is easily explained by the composition of Brazilian diet, which consists basically of rice and beans,¹⁸ and therefore poor in proteins of animal origin. By analyzing the survey on dietary frequency, we can observe that proteins of animal origin, good sources of copper and zinc, are seldom found. A study that assessed the intake of these minerals in female adolescents revealed an inadequate copper intake.¹⁹ Greger *et al.*²⁰ assessed the nutritional status in terms of iron, copper and zinc in 267 adolescents from Indiana/USA, aged between 11 and 15 years, and found out that the intake of zinc was equivalent to 75% of the recommended allowances; female adolescents consumed less than two thirds of the recommended amount. An inadequate iron intake was also observed among female

adolescents. Bergström *et al.*(1995)²¹ analyzed the diet of 731 Swedish adolescents, with mean ages of 14 to 17 years, and reported that iron intake was 1.6 times the recommended allowances for male adolescents and 0.9 times the recommended allowances for female adolescents. The results of iron intake we found are quite unfavorable for female adolescents, as they have not experienced menstrual losses yet and they will probably enter the reproductive age with low iron stores. Since these minerals are important for growth, their low intake is quite worrying.

The biochemical analysis showed that all adolescents had iron and zinc levels within the reference standard. With regard to iron intake, there is a similar report, but it does not consider the pubertal spurt.²² Male adolescents have a higher risk for iron deficiency during the initial adolescence, which corresponds to the rapid growth stage. Many female adolescents have a risk for iron deficiency later on, which probably results from iron loss due menstruation.²³ In the present study, the risks of deficiency at pubertal spurt seemed attenuated, as serum iron level was normal in the

Table 4 - Adequacy of iron, copper and zinc serum concentration (normal, low) in adolescents according to gender and reference values

Reference values	Male		Female	
	low n	normal n	low n	normal n
Iron (45-150 µg/dL)	0	19	0	28
Cooper (70-140 µg/dL)	1	18	1	27
Zinc (50-120 µg/dL)	0	19	0	28
Ferritin (<12 µg/l)	3	16	1	27

Table 5 - Iron, ferritin, copper and zinc serum concentration ($\mu\text{g/dL}$) in adolescents of both genders in the pubertal growth spurt

	Serum concentration ($\mu\text{g/dL}$)			
	Iron	Ferritin	Copper	Zinc
Male (n=19)	104.31 \pm 42.39 (45.0–226.2)	37.36 \pm 21.73 (10.5–91.6)	98.82 \pm 17.88 (80.0–125.0)	88.66 \pm 19.20 (65.0–132.5)
Female (n=28)	96.99 \pm 25.78 (50.0–156.4)	33.28 \pm 16.67 (11.7–83.8)	101.48 \pm 16.78 (50.0–142.5)	77.50 \pm 13.93 (60.0–132.5)

() variation of values

studied sample (Table 5). The highest muscle mass acquisition, with consequently more iron requirement, occurs at the peak growth spurt only in males, because in female adolescents, this occurs at menarche, that is, after the growth spurt. By considering the stages of pubertal spurt, Castillo-Durán *et al.*²⁴ have investigated the effect of zinc supplementation in Chilean adolescents with low idiopathic height, during one year, placing them in groups of impubescent (stage I) and pubescent (other stages) individuals. For both male and female adolescents who received placebo and were in puberty, the mean serum level of zinc was adequate. However, this study included adolescents that were still going to enter the growth spurt stage (males in G2), those who were actually at pubertal spurt (G3 and G4), and those who had already gone through

the spurt stage (G5). Another study that also considered the pubertal spurt did not report significant differences in the concentration of serum zinc of adolescents classified into Tanner stages as 1-2, 3 and 4-5 for both sexes. When grouped according to age, 13- and 14-year-old male adolescents showed a remarkable reduction of serum zinc, compared to other age groups. For this reason, the authors have suggested that male adolescents show an increased demand for zinc at the first stages of puberty. Even when adolescents were classified into Tanner stages, those ones at pubertal spurt were mixed with impubescent and post-puberty adolescents.²⁵

With regard to ferritin, three male adolescents and one female adolescent presented below-average values, possibly

Table 6 - Correlation* between body mass index – BMI, iron serum concentration ($\mu\text{g/dL}$), Ferritin (Fer), Cu and Zn and dietetic intake (mg) of Fe, Cu and Zn of adolescents of both genders in the pubertal growth spurt

Correlation	Adolescents	
	Male [†]	Female [‡]
BMI x Serum iron	0.260	-0.141
BMI x Serum copper	0.070	0.303
BMI x Serum zinc	0.180	-0.280
BMI x Ferritin	0.250	-0.096
Serum iron x Ferritin	0.178	0.006
Serum iron x Dietetic iron	-0.139	0.094
Serum copper x Dietetic copper	0.290	-0.760
Serum zinc x Dietetic zinc	-0.480	0.336
Serum iron x Ferritin	0.178	0.006

* Spearman correlation coefficient

† critical r value = 0.44

‡ critical r value = 0.38

due to the acquisition of muscle mass in males rather than in females during the spurt stage. The inadequate iron intake in 18 female adolescents, although the whole sample shows normal serum levels of iron and ferritin (Tables 4 and 5), may indicate that iron stores are being mobilized to maintain these levels, since the concentration of serum iron reflects the balance between absorbed iron, iron used for the synthesis of hemoglobin, iron released by the destruction of erythrocytes and the size of the deposit compartment, that is, it represents an accurate balance between the inflow and outflow of iron in the bloodstream.

The classification of adolescents according to BMI percentiles revealed that three female adolescents were obese (Table 3). Although representative studies on obese adolescents are rare, a multicenter study conducted in all Brazilian regions (north, northeast, midwest, southeast and south) assessed 13,715 adolescents aged between 10 and 19 years, and revealed a higher prevalence of overweight and obesity in female adolescents.²⁶ As the risk of obesity increases after menarche and obesity in adolescence tends to persist up to adulthood, the presence of obesity among adolescents at pubertal stage, that is, before menarche, is quite distressing.

No correlation was found between BMI and the serum levels of iron, ferritin, copper and zinc in males and females (Table 6), which probably indicates an increase in the intestinal absorption of iron in order to meet the demands of for this mineral during the pubertal spurt. It is important to remember that the growth hormone mobilizes iron stores and might be influencing these results. Laitinen *et al.*²⁷ have shown a positive correlation for copper at the stages before and after the pubertal spurt, which does not allow for a comparison between their results and ours. Antilla *et al.*²² have conducted for two years a longitudinal two-year study in Finland that included 60 male adolescents and have found that serum ferritin levels decreased during the two-year follow-up period, but they could not correlate iron stores with serum iron, or ferritin with BMI.

The serum levels of iron, copper and zinc showed no correlation with their dietary intake in both males and females, except for zinc, which showed a weak and negative correlation in male adolescents, with no statistically significant difference (Table 6). Other studies on the status of iron during adolescence correlated other parameters with iron intake. In Spain, Fernández-Ballart *et al.*²⁸ studied 302 healthy children aged between six months and 15 years, and have not found any correlation between the intake of nutrients and the biochemical parameters. Greger *et al.*²⁰ have not observed any correlation between the iron intake, hemoglobin and hematocrit levels in female adolescents.

Serum iron concentration seems to suffer other influences as important as those of dietary intake. It is well-known that anemia is the most prevalent nutritional disease on a worldwide basis, which begins in infancy and persists during adolescence; however, in Brazil, epidemiological studies of adolescents are few in comparison to the other

risk groups. In developing countries, the prevalence of anemia in adolescence has high rates, which range from 9 to 50%.²⁹ The interaction between minerals is extremely important so that they can be properly used by the body. Rodríguez-Matas *et al.*³⁰ have studied iron-deficient rats and have found that iron restriction after 30 to 40 days caused remarkable changes in the metabolism of copper and zinc.

Even though nine male adolescents and 12 female adolescents had an inadequate copper intake, the serum copper concentration was adequate in most adolescents (Tables 4 and 5). This could be explained due to the secretion of estrogen, which increases the serum copper levels.³¹ Klevay has suggested that the levels of serum copper are proportional to the level of circulating estrogen.³² Another explanation is the regulation mechanism of copper by the body, that is, when copper intake is low, the body protects itself from depletion by excreting small amounts; in other words, the efficiency of copper absorption varies inversely to copper intake.³³ Turlund *et al.*³⁴ have observed that, despite the low copper intake, adolescents had a positive balance. This suggests that the minimum limit set by the RDA is either too high or the marginal copper deficiency may be common but difficult to diagnose or that water supplies an additional amount of copper to meet the requirements or that these factors can be all combined.

Most adolescents, as shown in Table 1, showed inadequate dietary intake of zinc (15 males and 22 females), below the RDA; however, these adolescents presented normal serum levels of zinc. We know that serum zinc levels are low when dietary restriction is long lasting. Thus, the results observed herein are related to the body's ability to make homeostatic adjustments at different levels of zinc intake. The identification of zinc intake level below the RDA, at which the body is not able to compensate deficiency, does not seem to be an easy task. Therefore, zinc deficiency probably occurs due to some failure in the compensatory homeostatic response of the body or due to a low dietary intake for a long time. Zinc plays a crucial role in sexual maturation and growth, as already shown in a study of healthy primates,³⁵ which shows that dietary zinc may be a limiting factor to growth during adolescence. This way, primates at pubertal spurt which suffered zinc deficiency, but which had a normal intake of other nutrients, revealed sexual maturation and growth delay and reduction of serum zinc.

Although the intake of copper and zinc is below the RDA in adolescents, the traditional biochemical indicators of the dietary status of these minerals show normal values. It is not clear whether the recommended daily allowances are unnecessarily high or whether the biochemical indicators commonly used do not have the necessary sensitivity and specificity to assess the dietary status of these minerals. We do not know whether the serum levels of zinc and copper oscillate during growth or whether each individual has a considerably stable level of these minerals during the growth

stage, which indicates the necessity for population-based studies that compare the levels of iron, copper and zinc before and after the pubertal spurt in adolescents.

The literature, in general, classifies adolescents according to their age and not according to their physiological stage (sexual maturation). During the body changes that occur at the different stages of maturation, there are different nutritional requirements for each sex. In addition, iron, copper and zinc are transactional elements with strongly related biological interactions, and this can make us ponder whether iron supplementation is valid or not at this important growth stage.

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