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ORIGINAL COMMUNICATION

Copper and zinc intake and serum levels in patients with juvenile rheumatoid arthritis

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Objective: To evaluate the copper and zinc intake and serum levels in patients with juvenile rheumatoid arthritis (JRA), considering the pauci and polyarticular types, the disease activity and duration, the number of inflamed joints and the use of corticosteroids therapy.
Design: Cross-sectional study with control group.
Setting: Outpatients of the pediatric rheumatology public health clinic, of the Universidade Federal de São Paulo/Escola Paulista de Medicina, Brazil.
Subjects: Forty-one patients with JRA were evaluated and 23 patients' brothers, as a control group.
Interventions: Copper and zinc intake evaluation by Food Register method. Copper and zinc serum levels by atomic absorption spectrophotometry.
Results: The disease activity did not determine difference in copper ($P=0.624$) and zinc ($P=0.705$) intake, being predominantly below the Recommended Dietary Allowances. The serum copper in relation to control was statistically greater ($P=0.018$), showing that the number of inflamed joints is statistically significantly related with its variation ($P=0.001$). The serum zinc was not different either in relation to control ($P=0.940$) or to the disease characteristics.
Conclusions: The evaluation of copper intake seems to be of fundamental importance. It may influence the efficiency of the organic serum response. More research is needed to indicate, with security, adequate zinc intake.
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Keywords: juvenile rheumatoid arthritis; copper; zinc

Introduction

Copper and zinc physiology is considered a matter of great interest because of the possibility of being able to activate many key enzymes in the cellular metabolism. Copper and zinc are essential nutrients, and their daily intake is necessary to prevent diseases (World Health Organization, 1996). They are constituents of the superoxide-dismutase enzyme, which performs intracellular antioxidant functions (Tuncer *et al*, 1999). Copper is a constituent of ceruloplasmin, a powerful extracellular antioxidant enzyme (Halliwell & Gutteridge, 1990). The anti-inflammatory effects of copper and zinc have been documented in animals (Milanino *et al*, 1988) and in humans (Milanino *et al*, 1993). On the other hand, the acute or chronic inflammation induces metabolism alterations of these minerals, determining alterations in their serum and tissue levels (Soylak & Kirnap, 2001), as well as triggering other nutritional impairments (Gómez-Vaquero *et al*, 2001). However, there are few studies about the copper and zinc serum levels in juvenile rheumatoid arthritis (JRA; Makela *et al*, 1984; Honkanen *et al*, 1989; Bacon *et al*, 1990; Haugen *et al*, 1992; Strano *et al*, 1995).

Therefore, we proposed to evaluate the copper and zinc intake and serum levels in JRA patients, considering pauci and polyarticular types; the disease activity and duration; the number of inflamed joints and the use of corticosteroid therapy.

Methods

Forty-one outpatients, 21 females, with mean age of 11.3 y (range 3.3–17.8 y), of the Pediatric Rheumatology public health clinic, of the Universidade Federal de São Paulo,

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Escola Paulista de Medicina, São Paulo, SP, Brazil, with JRA were evaluated by a pediatric rheumatologist. The diagnosis of JRA was based on the American College of Rheumatology criteria (Brewer *et al*, 1977). Twenty-one patients presented active disease, eight of the pauciarticular type and 13 of the polyarticular type. Among the patients who did not present active disease, 11 were of the pauciarticular and nine of the polyarticular type.

The disease was considered active when there was at least one active joint and changes in the erythrocyte sedimentation rate (ESR). The disease duration was calculated by the relation between the time of the disease and the age of the patient, in years. The number of inflamed joints (range 1–12) included the active pauci and polyarticular types, totalling 21 patients. Corticosteroid therapy consisted of prednisone administration, with doses in the range 4–12.5 mg/day at the pediatric rheumatologist's criterion. Patients with and without the present use of this treatment were considered.

The control group was used for comparison of the Cu and Zn serum levels and constituted 23 patients' brothers, 13 female, with mean age of 9.9 y (range 4.7–17.2 y). The inclusion criterion was the absence of acute or chronic disease.

Consent was obtained in writing from parents, and the Ethics Committee in Research of the Universidade Federal de São Paulo/Hospital São Paulo approved the study.

Dietary evaluation

A four-consecutive-days food register (Karkek, 1987) method was used, including one weekend. The copper and zinc intake was calculated using the validated and standardized Virtual Nutri-USP (Philippi *et al*, 1996) software.

The calculated dietary intake was compared with the Recommended Dietary Allowances (RDA; National Research Council, 1989).

Biochemical analysis

Blood samples were collected with patients fasting, between 8:00 and 9:00 a.m. The serum samples were kept at $-18^{\circ}C$ until assay. The serum samples were diluted with distilled and deionized water from the Milli-Q-Plus System (Millipore Corp.) in a 1:10 proportion (Whitehouse *et al*, 1982), and the copper and zinc concentrations were determined by atomic absorption spectrophotometry (Perkin-Elmer, model 5.100), under the following conditions: wavelength 324.8 nm (Cu) and 213.9 nm (Zn); slit 0.7 nm; air acetylene oxidant flame; energy 71 (Cu) and 65 (Zn); duplicate reading with integration time of 2 s. Stock standard solutions of 1000 mg metal/l (Perkin-Elmer, PEN 4300183 for Cu and PEN 9300178 for Zn) were diluted with glycerol at 10% (Cu) and glycerol at 5% (Zn), resulting in working standard solutions containing 0.1, 0.3, 0.5, 1.0 and 2.0 mg Cu or Zn/l. Accuracy was monitored by comparison with the commercial Quality Control Standard 21 (Perkin-Elmer, PEN 9300281) for both elements. Results are expressed as μg Cu or Zn/dl.

All chemicals used were of analytical grade. Acid-washed glassware was used throughout the study.

Statistical analysis

Student's *t*-test for independent samples (Bussab & Morentin, 1987) was used for comparison of means between the evaluated groups. Pearson's linear correlation coefficient (Bussab & Morentin, 1987) was used for the quantification between the observed numerical variables. Covariance analysis (Neter *et al*, 1996) was used to compare the serum levels, controlled for age. Multiple linear regression (Neter *et al*, 1996) was used to verify the significance of the disease characteristics in the copper and zinc serum levels. An alpha confidence level of 5% was adopted. The results are expressed as means and standard deviations.

Results

Dietary evaluation

No intake differences were observed between the control and JRA groups, respectively 1.29 ± 0.70 and 1.21 ± 0.60 for copper ($P = 0.644$) and 8.12 ± 3.30 and 8.19 ± 3.18 for zinc ($P = 0.938$). In both groups the mean copper and zinc intakes were below the recommendation.

Daily mean intake of zinc (Table 1) did not show statistical differences in patients with and without disease activity ($P = 0.705$), not reaching the RDA recommendation in any of the groups. Regarding copper, the mean intake also did not show significant differences between the compared groups ($P = 0.624$). Only 13 patients reached the safe and adequate range of the recommended copper intake, with a mean intake of 1.74 ± 0.76 mg, which was statistically higher than the mean of the other 28 patients, 0.97 ± 0.31 mg ($P = 0.003$).

Also, there was no statistical difference between male and female patients with disease activity ($P = 0.774$ for copper and $P = 0.667$ for zinc) and without disease activity ($P = 0.656$ for copper and $P = 0.930$ for zinc; Table 2).

Table 1 Daily copper and zinc intake, determined by a 4 day food register in JRA patients with and without disease activity

	JRA			
	Activity (n = 21)	*No Activity (n = 20)*	*RDA*[a]	P
Copper (mg)	1.15 ± 0.57 (0.6–3.2)	1.24 ± 0.64 (0.6–3.2)	[b] —	0.624
Zinc (mg)	8.42 ± 3.7 (1.1–16.3)	8.04 ± 2.5 (3.1–12.9)	10–15	0.705

[a]RDA, recommended dietary allowances.
[b]4–6 y = 1.0–1.5 mg Cu; 7–10 y = 1.0–2.0 mg Cu; ≥11 y = 1.5–2.5 mg Cu.
P, descriptive level of Student's *t*-test.

Table 2 Daily copper and zinc intake in male and female JRA patients with and without disease activity

	JRA			
	Activity		*No activity*	
	Male (n = 10)	*Female (n = 11)*	*Male (n = 10)*	*Female (n = 10)*
Copper (mg)	1.19 ± 0.29 (0.9 – 1.9)	1.12 ± 0.76 (0.3 – 3.2)	1.31 ± 0.73 (0.7 – 3.2)	1.18 ± 0.59 (0.6 – 1.4)
	$P = 0.774$		$P = 0.656$	
Zinc (mg)	8.70 ± 4.83 (1.1 – 16.3)	7.98 ± 2.71 (3.7 – 12.6)	8.10 ± 2.19 (5.2 – 12.6)	7.99 ± 2.90 (3.1 – 12.9)
	$P = 0.667$		$P = 0.930$	

P, descriptive level of Student's *t*-test.

Biochemical evaluation

As the JRA and control groups were not similar in relation to gender distribution, the serum copper and zinc means were first compared according to this variable, in each group (Table 3). In the JRA patients group, there were no significant differences in sex related to copper ($P = 0.067$) and zinc ($P = 0.091$) serum levels. The same was observed in the control group subjects for copper ($P = 0.927$), as well as for zinc ($P = 0.298$).

When the copper and zinc serum levels were compared by sex between JRA and control groups (Table 4), only the JRA group of males showed higher copper levels than the control males ($P = 0.004$).

The comparison between the JRA and the control groups, controlled for age, showed higher serum copper levels in the JRA group ($P = 0.018$) and without statistical significant difference for the zinc level ($P = 0.940$; Table 5).

When copper and zinc levels were compared considering separately the disease activity, the pauci and polyarticular types, the use of corticosteroid, the disease duration and the number of inflamed joints (Table 6), it was verified that only the disease activity and the number of inflamed joints have showed statistical significance with copper levels, presenting descriptive levels of 0.012 and 0.001, respectively.

Table 3 Serum copper and zinc comparison between gender of the JRA and control groups

	JRA		*Control*	
	Male (n = 20)	*Female (n = 21)*	*Male (n = 10)*	*Female (n = 13)*
Copper (μg/dl)	154.0 ± 35.7 (105 – 225)	133.5 ± 33.5 (90 – 235)	125.5 ± 13.8 (110 – 150)	126.5 ± 33.0 (80 – 190)
	$P = 0.067$		$P = 0.927$	
Zinc (μg/dl)	89.7 ± 17.5 (65 – 125)	97.6 ± 10.2 (85 – 115)	90.0 ± 12.0 (70 – 110)	95.0 ± 10.4 (80 – 110)
	$P = 0.091$		$P = 0.298$	

P, descriptive level of Student's *t*-test.

Table 4 Serum copper and zinc comparison between JRA and control groups according to gender

	Male		*Female*	
	JRA (n = 20)	*Control (n = 10)*	*JRA (n = 21)*	*Control (n = 13)*
Copper (μg/dl)	154.0 ± 35.7 (105 – 225)	125.5 ± 13.8 (110 – 150)	133.5 ± 33.5 (90 – 235)	126.5 ± 33.0 (80 – 190)
	$P = 0.004$		$P = 0.555$	
Zinc (μg/dl)	89.7 ± 17.5 (65 – 125)	90.0 ± 12.0 (70 – 110)	97.6 ± 10.2 (70 – 110)	95.0 ± 10.4 (80 – 110)
	$P = 0.968$		$P = 0.476$	

P, descriptive level of Student's *t*-test.

Table 5 Serum copper and zinc of subjects in JRA and control groups, controlled for age

	JRA (n = *41*)	*Control* (n = *23*)	P
Copper (μg/dl)	143.5 ± 35.7 (90 – 235)	126.0 ± 25.9 (80 – 190)	*P* = 0.018
Zinc (μg/dl)	93.7 ± 14.6 (65 – 125)	92.6 ± 11.3 (70 – 110)	*P* = 0.940

P, descriptive level of Student's *t*-test.

Table 6 Serum copper and zinc of patients with JRA, according to the activity and type of disease, use of corticosteroid, disease duration and number of inflamed joints

	Copper (μg/dl)	*Zinc (μg/dl)*
Activity[a]		
Activity (*n* = 21)	156.9 ± 39.6	92.1 ± 16.6
No activity (*n* = 20)	129.5 ± 25.1	95.5 ± 12.3
	P = 0.012*	*P* = 0.469
Type[a]		
Pauciarticular (*n* = 19)	136.8 ± 37.6	94.4 ± 10.5
Polyarticular (*n* = 22)	149.3 ± 33.8	93.1 ± 17.6
	P = 0.270	*P* = 0.774
Corticosteroid therapy[a,b]		
Yes (*n* = 8)	163.1 ± 36.7	89.3 ± 21.1
No (*n* = 5)	159.0 ± 26.3	88.0 ± 15.2
	P = 0.832	*P* = 0.902
Duration of disease[c] (*n* = *41*)		
Coefficient	−0.011	0.214
P	0.946	0.178
Number of inflamed joints[c](*n* = *21*)		
Coefficient	0.494	−0.297
P	0.001*	0.059

[a]Student's *t*-test for independent samples.
[b]Pearson's linear correlation coefficient.
[c]Polyarticular type patients.
*Significant difference.

The study of the combined relation between the level of serum copper and the variables activity, type and disease duration, use of corticosteroid and the number of inflamed joints, from the multiple linear regression (Table 7), showed that only the number of inflamed joints varies statistically significantly with serum copper level ($P = 0.001$).

Table 7 Multiple linear regression of the relation between serum copper level and the studied variables[a]

	Coefficients	*Standard error*	P
Constant	129.774	6.259	< 0.001
Number of inflamed joints	5.038	1.419	0.001

Dependent variable, serum copper.
[a]Variables: activity, type, disease duration, corticosteroid therapy and number of inflamed joints.

Discussion

Copper and zinc intake

It seems that JRA characteristics do not determine significant differences in copper and zinc intake. Our results (Table 1) confirm previous work (Bacon *et al*, 1990), and the type of disease (pauci/poly/systemic) reported by other authors (Bacon *et al*, 1990; Haugen *et al*, 1992). Nevertheless, the majority of individuals of the control group and of the JRA patients, with and without disease activity, did not reach the RDA recommendation for both elements. Intakes below the recommendation for healthy children are reported in many regions, such as the USA (Hambidge *et al*, 1985), UK (World Health Organization, 1996), Canada (Gibson *et al*, 1989), Papua-New Guinea (Ross *et al*, 1986) and Brazil (Shrimpton, 1984). The nutrient intake recommendations are intended for healthy people and any type of disease may change the specific nutrient needs. However, JRA subjects as well as healthy children presented lower than recommended intakes of copper and zinc. However, for patients, this may signify a greater deficit, as there may be an increase in the requirements for those nutrients caused by the inflammatory process (Haugen *et al*, 1992) and by the specific functions that copper and zinc have in inflammatory diseases (Rosenstein & Caldwell, 1999).

Serum copper

As there was no statistical difference in the serum copper and zinc levels between sex (Table 3), the male and female subjects of the JRA and control groups were considered together.

Our results regarding the increased serum copper level in the JRA group in relation to the control group, controlled for age (Table 5), are consistent with previous studies (Bacon *et al*, 1990; Haugen *et al*, 1992; Strano *et al*, 1995). Ninety percent of the serum copper is incorporated to the ceruloplasmin (Rosenstein & Caldwell, 1999), which is one of the main extracellular antioxidants (Kiziltunc *et al*, 1998). Therefore, the increase of serum copper may be due to the increase of the ceruloplasmin, which is an acute phase protein (Conforti *et al*, 1983), whose role in adjuvant arthritis is to neutralize free oxygen radicals, mainly anion superoxide (Zoli *et al*, 1998) in an attempt to stop the process of turning chronic.

The deficient copper intake, observed in our patients, which may lead to reduced intracellular copper and thus to reduced defense against free radicals (Haugen *et al*, 1992), probably did not damage the acute phase response to the inflammatory process. This probably occurred because the low copper intake was not enough to deplete the hepatic reserves (Honkanen *et al*, 1991b), thus not impeding the adequate synthesis of ceruloplasmin. There are two possible explanations for this hypothesis. First, copper used to increase ceruloplasmin synthesis could derive from changes in the balance between intestinal absorption and excretion of copper. Second, copper could come from other areas of

the organism, such as the kidneys and the red cells (Conforti *et al*, 1983).

It is known that the cerulosplamin correlates with JRA (Harvey *et al*, 1987) and rheumatoid arthritis (RA; Kiziltunc *et al*, 1998) activity, explaining the result of the significantly higher serum copper during the disease activity (Table 6). The mean serum copper of patients without disease activity, 129.5 μg/dl, is within the normal serum values mentioned in the literature (World Health Organization, 1996) and normal value is expected in periods with no disease activity (Milanino *et al*, 1985).

Serum copper did not show statistical difference between the pauci and polyarticular types (Table 6), nevertheless, the latter was greater than that of the control; $P=0.013$ (not shown). These results agree with those of Bacon *et al* (1990) when comparing polyarticular patients with healthy children. When the comparison is between the pauci and polyarticular types, our results are not in accordance with those of Haugen *et al* (1992). However, this difference may be due to the fact that, in the study of those authors, only two patients were using corticosteroids. If we consider that the mineral bone density is diminished in the polyarticular type, with and without the disease activity (Cassidy & Petty, 1995), we can raise the hypothesis of Cu mobilization from bone: once in the tissue, the mineral is probably found adsorbed to the crystals (Hilário *et al*, 1991). This could also help to explain the increased serum copper levels in JRA, particularly in the polyarticular type.

The polyarticular group, as expected, presented serum copper levels above normal values, although without statistical difference due to the treatment with corticosteroids (Table 6). Several authors have reported diminished levels of copper during this treatment (Scudder *et al*, 1978a; Brown *et al*, 1979). The decrease of serum copper could be due to the use of corticosteroids, explaining the similar copper levels found in patients not using corticosteroid therapy. However, these results were obtained from a small number of observations, which could not represent reality, prohibiting further discussions.

The disease duration did not show correlation with the serum copper levels, even when the disease duration was considered related to the age of the patient, as in this study (Table 6), or when the duration of arthritis over 1 y was considered (Honkanen *et al*, 1989), or the mean of 8.6 y (Balogh *et al*, 1980).

The number of inflamed joints, besides correlating positively with serum copper level (Table 6), was the only disease characteristic that showed statistical significance on the variation of this level (Table 7). A total of 65 inflamed joints in 12 male patients and a total of 47 in nine female patients were observed. It was also observed that the mean serum copper was not different between JRA and control female subjects, while the mean serum copper of JRA males was significantly higher than the mean of control males (Table 4). Considering these results, the group of JRA male alone could account for the difference of serum copper between JRA and control groups, and this fact is probably due to the higher number of inflamed joints observed in this group. In the RA subjects, increased levels of serum copper are accompanied by high levels of copper in the synovial fluid (Biemond *et al*, 1984), possibly meaning acceleration of the lgG aggregates formation (Gerber, 1974) that occur in the synovial fluid of these patients (Winchester *et al*, 1970), resulting in local articular ceruloplasmin protection against oxidative damage (Scudder *et al*, 1978b; Kiziltunc *et al*, 1998). Another possibility is that it may signify tissue damage by the stability reduction of cellular membrane (Chvapil *et al*, 1972). This damage could occur due to the lack of capacity of the tissues to use the extra copper because of the high ceruloplasmin blood level or due to blocked copper receptors. In this way, there is a situation in which tissues are actually deficient in copper, a situation that is known to cause defects in the structural stabilization of fibrous proteins of the connective tissues and also lead to the diminished tensile strength of elastin and collagen (Hannson *et al*, 1975). For some authors, the increased serum copper in JRA reflects the antioxidant activity, with possible protective effects, especially in the presence of tissue damage or destruction (Al-Timimi and Dormandy, 1977; Kiziltunc *et al*, 1998); this increase may signify an important component of the systemic inflammatory response (Scudder *et al*, 1978a).

Serum zinc

The serum zinc study of the JRA group did not show statistically significant difference, either in relation to the control group (Table 5), or in relation to the disease characteristics (Table 6), the serum level always being within the range of normal values, 80–110 μg/dl, mentioned in the literature (World Health Organization, 1996).

The unchanged serum zinc level under inflammatory conditions is in accordance with the results of Aaseth *et al* (1978) and of Matarran-Perez *et al* (1989). Nonetheless, increased (Hannson *et al*, 1975) or decreased (Moretti *et al*, 1988; Tuncer *et al*, 1999; Soylak & Kirnap, 2001) serum levels have also been observed, possibly due to the differences in the studied patients, in the established treatment and in the chosen fluids for the analysis of that element.

During inflammatory processes, zinc increase seems to occur in the liver and also in the injured tissue, such as synovial fluid of arthritic patients. As zinc is a cofactor for the protein and nucleic acid synthesis, it is conceivable that a portion of the accumulated zinc in the liver is involved in the increased synthesis of acute phase proteins (Powanda *et al*, 1973). The interleukin-1, produced by stimulated macrophages, increases metallothionein-mediated hepatic uptake (Honkanen *et al*, 1991a). The increased metallothionein synthesis is necessary for the protein synthesis of the acute phase (Oliva *et al*, 1987). In RA, decreased absorption and hepatic accumulation were described, after a zinc tolerance test (Naveh *et al*, 1997).

Some results in the literature may explain normal or decreased zinc levels, during inflammation. Thus, the significant correlation between serum decrease and hepatocyte increase may signify that this increase occurs at serum depletion cost (Oliva *et al*, 1987; Zoli *et al*, 1998). Serum albumin, which is an important Zn-carrier, is decreased during inflammation, and presents positive correlation with the reduced zinc levels (Milanino *et al*, 1993; Maes *et al*, 1997). Therefore, zinc absorption and distribution seems to be disturbed in JRA (Honkanen *et al*, 1989), implying that its serum level is probably, one of the nonspecific features of inflammation (Balogh *et al*, 1980; Honkanen *et al*, 1991a).

Corticosteroid treatment did not determine alteration in the serum zinc (Table 6). Several results confirm that the use of these drugs does not significantly influence serum value of this mineral (Peretz *et al*, 1989; Milanino *et al*, 1993). It is known that corticosteroids increase zinc transport to the membranes (Chvapil, 1976), as well as to urine (Ellul-Micallef *et al*, 1976), zinc being, possibly, a mediator of the membrane-stabilizing effect of the steroids (Chvapil, 1976).

Conclusions

Considering the important role of the serum copper increase in inflammatory response, the evaluation and orientation of copper intake seems to us of fundamental importance, as this may have influence in the organic serum response efficiency.

In relation to zinc, more research is required to determine, with security, adequate zinc intake.

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