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Serum Copper and Zinc Levels in Healthy Greek Children and Their Parents

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Abstract The aim of this study was to investigate whether there is a correlation between copper (Cu) and zinc (Zn) levels in children and their parents, considering their nutritional habits. Cu and Zn concentrations were measured by flame atomic absorption spectrophotometry in the serum of 66 healthy children, aged 3–14 years, and their parents, residing in a region of Greece (Thrace). Cu levels were higher in mothers than those in fathers, but they were lower in both parents than those in children. They also tended to decrease with age in both parents and children, whereas Zn levels significantly increased with age in children. There was a positive correlation between children's and mothers' Zn levels, as well as children's and both parents' Cu levels. Children used to eat meat, fish, vegetables, and legumes as frequently as their parents, but they were consuming more eggs, milk, and fruits than the latest. Regarding parents' diet, higher Zn levels were depended on the consumption of meat and milk, whereas higher Cu levels were depended on the consumption of milk. Consequently, children's Cu and Zn levels are related to their parents' levels, which can be influenced by their nutritional habits.

Keywords Children · Copper · Nutrition · Parents · Zinc

Introduction

Copper (Cu) and zinc (Zn) are essential trace elements involved in many biological processes necessary to maintain life. As complexes with proteins or as cofactors, they are

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associated with various enzyme systems functioning in cellular metabolism and reproduction and in membrane integrity. Therefore, their deficiency represents a hazard for human health. Furthermore, according to the declaration of nutrition, health and intelligence for the child-to-be, Zn and Cu are two of the many nutrients implicated in healthy reproduction and lifelong health [1].

Zn plays important roles in growth and development, immune response, neurological function, and reproduction. On the cellular level, Zn's function can be divided into three categories [2]: (1) catalytic (more than 300 enzymes require Zn for their activities) [3], (2) structural (role in the structure of proteins—Zn finger motif—and cell membranes) [3–5], and (3) regulatory (role in gene expression, transcription, cell signaling, hormone release, apoptosis) [6].

Cu is involved in electron transport and this ability explains its role in oxidation–reduction reactions. It is a functional component of a number of essential enzymes and some copper-dependent functions are energy production [7], connective tissue formation [8], iron metabolism [8, 9], metabolism and synthesis of neurotransmitters [9, 10], melanin formation [8], antioxidant functions [7, 8, 11], and regulation of gene expression [7].

The deficiency of these trace elements is due to malnutrition or malabsorption or poor bioavailability. The most susceptible individuals to Zn or Cu deficiency are infants and children, pregnant and lactating women, patients receiving total parenteral nutrition, the elderly, strict vegetarians, individuals with severe or persistent diarrhea, malabsorption syndromes, and inflammatory bowel disease [3, 4, 8, 10]. The World Health Organization/United Nations Children's Fund recommends that all children with an acute diarrheal illness should be treated with zinc, regardless of etiology [12]. The clinical signs of Zn deficiency are the slowing or cessation of growth and development, delayed sexual maturation, characteristic skin rashes, chronic and severe diarrhea, immune system deficiencies, impaired wound healing, diminished appetite, impaired taste sensation, night blindness, and behavioral disturbances [4, 13], whereas of Cu deficiency are anemia, neutropenia, osteoporosis, pigmentation, impaired growth, and neurological signs [7, 8].

In our previous study [14], we had noticed significant correlations between food intake and Cu and Zn levels in healthy children living in Greece. The aim of this study was to identify the relation between Cu and Zn levels in children and their parents, in addition to the parental influence on children's nutritional habits which affect Cu and Zn concentrations. Age and gender were also taken into account for the levels of these bioelements.

Subjects and Methods

The study population consisted of 66 children and their parents. More specifically, it comprised of 24 boys and 42 girls, aged 7.53±3.31 years (mean±standard deviation (SD); range 3–14 years), and 66 men and 66 women, aged 34.83±6.88 years (range, 22–56 years). Children were those that were examined in our clinic for routine medical examination during a 6-month period (from March to September 2008), and they were considered healthy according to the following criteria: (1) absence of any acute or chronic pathology clinically evident at the moment of examination and (2) normal blood chemistry, hematology, and urine analysis. All children and their parents were residing in urban areas in Thrace, a northeast region of Greece, and all belonged to middle class families.

The metals (Cu and Zn) were measured in the serum of the children and their parents using flow injection analysis–flame atomic absorption spectrophotometry with a Perkin-

Elmer 2360 spectrophotometer. Children's and parents' age was reported in years and categorized as ≤5, 6–10, and >10 years and <30, 30–39, and ≥40 years, respectively.

The daily nutritional intake of each child and parent was evaluated by a 1-week dietary protocol filled in by both parents in combination with a final 24-h dietary recall interview made by the doctor. The amounts of their daily nutrients were then calculated according to a coded food list.

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS), version 11.0 (SPSS, Inc., Chicago, IL, USA). Continuous variables were expressed as the mean±SD and categorical variables as frequencies and percentages. A Kolmogorov–Smirnov test for normality was performed and data were analyzed using the Student's *t* test and one-way analysis of variance (ANOVA), followed by Tukey's test to assess differences of a continuous variable between two or more groups of children or parents, respectively. Pearson's *r* correlation coefficient was used to assess the relation between two continuous variables, whereas Cohen's κ coefficient as a measure of agreement between children and parents. All tests were two tailed and statistical significance was assigned for *p* values less than 0.05.

Results

In our study, children's Zn levels ranged from 68 to 144 μg/dl with a mean level of 99.88± 19.08 μg/dl (median 99.5 μg/dl), whereas parents' Zn levels ranged from 63 to 159 μg/dl with a mean level of 98.99±18.54 μg/dl (median 97 μg/dl). Children's Cu levels ranged from 108 to 254 μg/dl, with a mean level of 167.02±35.07 μg/dl (median 172.5 μg/dl), while parents' Cu levels ranged from 88 to 219 μg/dl with a mean level of 147.10±33.11 μg/dl (median 130 μg/dl).

There was no statistically significant difference between boys and girls, either in the Zn levels (boys 101.21±20.27 μg/dl vs girls 99.12±18.58 μg/dl; *t* test, $p=0.672$) or in the Cu levels (boys 163.54±32.96 μg/dl vs girls 169.00 ±36.46 μg/dl; *t* test, $p=0.547$). As for the parents, there was also no effect of gender on the Zn levels (men 98.68±15.62 μg/dl vs women 99.30±21.18 μg/dl; paired samples *t* test, $p=0.834$). On the contrary, Cu levels were significantly higher in women than in men (women 151.27±32.29 μg/dl vs men 142.92± 33.64 μg/dl; paired samples *t* test, $p=0.015$).

The levels of Zn in relation to children's age and sex are given in Table 1. A one-way ANOVA revealed a statistically significant effect of age on the levels of Zn in the total of children (one-way ANOVA, $p=0.012$) as well as in boys (one-way ANOVA, $p=0.004$), whereas there was no significant correlation between age and Zn levels in girls (one-way ANOVA, $p=0.559$). In particular, Tukey's test revealed that Zn levels in children >10 years old were significantly higher than in younger children (total of children: ≤5 vs >10 years: $p=0.012$, 6–10 vs >10 years: $p=0.049$, ≤5 vs 6–10 years: $p=0.744$, Tukey's honestly significant differences (HSD) test; boys: ≤5 vs >10 years: $p=0.027$, 6–10 vs >10 years: $p=0.006$, ≤5 vs 6–10 years: $p=0.997$, Tukey's HSD test). In the first two age intervals, there was no significant difference between Zn levels in boys and girls, while in children >10 years old, Zn levels were higher in boys than those in girls (boys 121.44±15.56 μg/dl vs girls 101.38±14.47 μg/dl; *t* test, $p=0.015$).

The levels of Cu in relation to children's age and sex are also given in Table 1. A one-way ANOVA showed a statistically negative correlation between age and Cu levels in the total of children (one-way ANOVA, $p=0.001$), in boys (one-way ANOVA, $p=0.035$) and in girls (one-way ANOVA, $p=0.014$). In particular, Tukey's test revealed that Cu levels in

Table 1 Zn and Cu Levels (micrograms per deciliter; mean±SD) in Relation to Children's Age and Sex

Age (years)	Zn				Cu			
	Total of children	Boys	Girls	p value[b]	Total of children	Boys	Girls	p value[b]
≤5	95.27± 17.36	96.80± 16.87	94.82± 17.99	0.892	184.91± 29.98	189.80± 25.10	183.47± 31.82	0.689
6–10	98.96± 17.35	96.20± 15.40	100.59± 18.65	0.536	160.48± 31.13	152.80± 24.96	165.00± 34.15	0.335
>10	112.00± 17.87	121.44± 15.56	101.38± 14.47	0.015	146.47± 29.70	151.56± 24.96	165.00± 34.15	0.472
p value[a]	0.012	0.004	0.559		0.001	0.035	0.014	

[a] *p* value (one-way ANOVA) for the comparison between age intervals

[b] *p* value (*t* test) for the comparison between boys and girls

children ≤5 years old were significantly higher than those in older children (total of children: ≤5 vs >10 years: p=0.001, 6–10 vs >10 years: p=0.303, ≤5 vs 6–10 years: p=0.019, Tukey's HSD test; boys: ≤5 vs >10 years: p=0.045, 6–10 vs >10 years: p=0.994, ≤5 vs 6–10 years: p=0.049, Tukey's HSD test; girls: ≤5 vs >10 years: p=0.011, 6–10 vs >10 years: p=0.207, ≤5 vs 6–10 years: p=0.238, Tukey's HSD test). There was no statistically significant difference between Cu and Zn levels in boys and those in girls in none of the three age intervals.

Zn levels in relation to parents' age and gender are given in Table 2. There was no statistically significant effect of age on Zn levels, either in men (one-way ANOVA, p=0.571) or in women (one-way ANOVA, p=0.807) or in the total of adults (one-way ANOVA, p=0.590). Furthermore, no significant difference was noted between Zn levels in men and those in women.

On the other hand, a one-way ANOVA revealed a significant negative correlation between age and Cu levels in men (one-way ANOVA, p=0.013), in women (one-way ANOVA, p=0.014) and in the total of adults (one-way ANOVA, p<0.001). As we can also see in Table 2, Cu levels tended to decrease as parents were getting older. More specifically,

Table 2 Zn and Cu Levels (micrograms per deciliter; mean±SD) in Relation to Parents' Age and Sex

Age (years)	Zn				Cu			
	Total of parents	Men	Women	p value[b]	Total of parents	Men	Women	p value[b]
<30	96.94± 18.31	94.67± 19.12	97.76± 18.34	0.671	167.44± 34.14	172.33± 37.10	165.68± 33.63	0.624
30–39	98.75± 16.45	98.12± 13.71	99.41± 19.07	0.756	142.17± 30.31	140.67± 31.67	143.72± 29.27	0.688
≥40	101.58± 22.56	100.96± 17.00	103.22± 34.64	0.855	135.85± 28.79	135.00± 30.19	138.11± 26.22	0.787
p value[a]	0.590	0.571	0.807		<0.001	0.013	0.014	

[a] *p* value (one-way ANOVA) for the comparison between age intervals

[b] *p* value (*t* test) for the comparison between men and women

Cu levels in adults <30 years old, which were not different from children's Cu levels (men vs children: $p=0.422$; women vs children: $p=0.725$; total of adults vs children: $p=0.952$), were significantly higher than those in adults 30–39 years old (men: $p=0.028$; women: $p=0.025$; total: $p<0.001$), or those in adults ≥40 years old (men: $p=0.011$; women: $p=0.041$; total: $p<0.001$). There was no significant difference between the other two age intervals (men 30–39 vs ≥40 years old: $p=0.786$; women 30–39 vs ≥40 years old: $p=0.879$, total 30–39 vs ≥40 years old: $p=0.606$). Furthermore, there was no statistically significant relation between Cu levels and parents' gender in none of the age intervals.

Zn and Cu levels in children and their parents in relation to their gender are given in Table 3. There was no statistically considerable difference between children's and parents' Zn levels in none of the groups. On the other hand, Cu levels were higher in children than those in their parents in all groups.

We noted a statistically significant strong relation between Zn levels in the total of children and those in their mothers (Pearson's $r=0.433$, controlling for age; $p<0.001$), but no relation was shown between children's Zn levels and their fathers' (Pearson's $r=0.037$, $p=0.770$). These results are demonstrated in Fig. 1. A positive correlation was also observed between Zn levels in girls and those in their mothers (mother: Pearson's $r=0.586$, $p<0.001$; father: $r=0.029$, $p=0.859$), but no significant correlation was revealed between Zn levels in boys and those in their parents (mother: Pearson's $r=0.268$, $p=0.217$; father: $r=0.134$, $p=0.541$).

On the contrary, there was a statistically considerable strong positive correlation between Cu levels in the total of children and those in both parents (mother: Pearson's $r=0.551$, $p<0.001$; father: $r=0.486$, $p<0.001$), relation that is demonstrated in Fig. 2. A positive association was also observed between Cu levels in girls and those in their parents (mother: Pearson's $r=0.587$, $p<0.001$; father: $r=0.528$, $p<0.001$) as well as between Cu levels in boys and those in their parents (mother: Pearson's $r=0.524$, $p=0.010$; father: $r=0.405$, $p=0.045$).

In our previous study [14], we had proved significant correlations between Cu and Zn levels in Greek children and food intake. In this study, we investigated the relation between Cu and Zn levels in parents and their nutritional habits. The results are given in Table 4. A one-way ANOVA showed a statistically significant effect of the consumption of meat and milk on the Zn levels. In detail, the consumption of meat was the strongest determinant of Zn levels, which statistically significantly increased as the former was increasing (six to seven times per week: $p=0.003$, three to five times per week: $p=0.028$, less than or equal to two times per week: $p=0.002$). Zn levels did not significantly differ between the other two groups ($p=0.220$). In addition, a positive correlation was found between Zn levels and the consumption of milk, where significantly higher levels of Zn were noted in parents who used to drink milk six to seven times per week (Zn levels: milk consumption less than or

Table 3 Zn and Cu Levels (micrograms per deciliter; mean±SD) in Children and their Parents in Relation to their Gender

	Zn			Cu		
	Children	Adults	p value	Children	Adults	p value
Men	101.21±20.27	98.68±15.62	0.534	163.54±32.96	142.92±33.64	0.011
Women	99.12±18.58	99.30±21.18	0.963	169.00±36.46	151.27±32.29	0.009
Total	99.88±19.08	98.99±18.54	0.754	167.02±35.07	147.10±33.11	<0.001

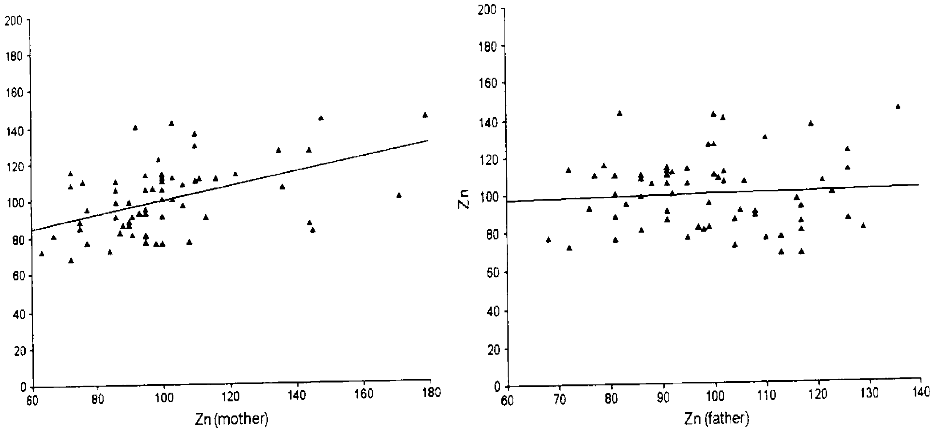

Fig. 1 Children's Zn levels in relation to their parents' Zn levels

equal to five times per week 95.03±15.04 vs six to seven times per week 102.39±20.59; *t* test, $p=0.019$). No statistically significant association was found between Zn levels and the consumption of fish, green vegetables, legumes, eggs, and fruits.

As for the Cu levels, they tended to increase as the consumption of milk was increasing (Cu levels: milk consumption less than or equal to five times per week 140.69±33.58 vs six to seven times per week 152.61±31.92; *t* test, $p=0.039$). There was also a marginal increase in the levels of Cu as the consumption of fish was increasing ($p=0.083$), while the interaction effect between Cu levels and the consumption of meat ($p=0.328$), green vegetables ($p=0.602$), legumes ($p=0.787$), eggs ($p=0.137$), and fruits ($p=0.137$) was not statistically significant.

Moreover, we studied the relation between children's and parents' nutritional habits. For this reason, we calculated the total percentage of cases where we had an agreement of the nutritional habits and Cohen's κ agreement coefficient.

A strong agreement was observed between children and mothers in the consumption of red meat/chicken (total percentage of agreement=75.8%, $\kappa=0.566$, $p<0.001$), fish (83.4%, $\kappa=0.720$, $p<0.001$), green vegetables (81.9%, $\kappa=0.645$, $p<0.001$), and legumes(81.9%, $\kappa=0.571$, $p<0.001$), whereas a moderate one was found in the consumption of milk

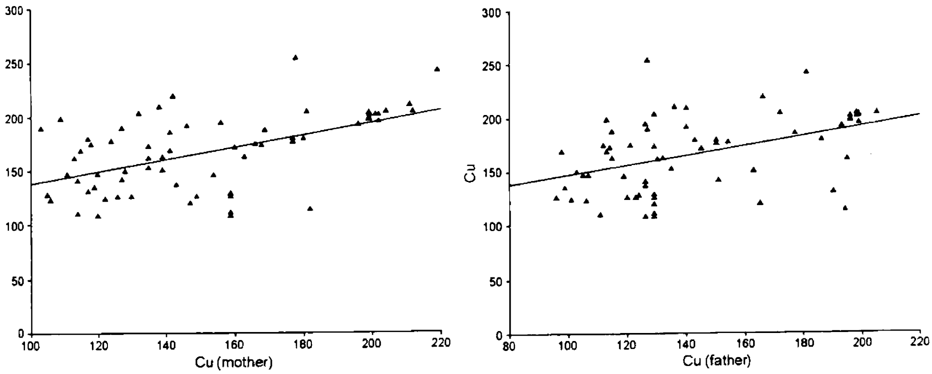

Fig. 2 Children's Cu levels in relation to their parents' Cu levels

Table 4 Serum Zn and Cu Levels (micrograms per deciliter; mean±SD) of Parents in Thrace in Relation to their Nutritional Habits

	Zn	Cu
Red meat/chicken		
≤2 times/week	92.14±17.69	141.28±32.82
3–5 times/week	98.56±17.17	150.52±34.45
6–7 times/week	109.59±20.51	142.18±27.65
p value	0.003	0.328
Fish		
Never	97.35±17.31	135.82±33.71
Rarely	99.57±18.85	142.32±28.64
≥1 time/week	99.03±18.86	152.76±34.79
p value	0.917	0.083
Green vegetables		
Never	96.00±15.73	139.93±33.43
1–2 times/week	96.88±17.02	145.30±33.80
≥3 times/week	100.31±19.56	148.98±32.98
p value	0.547	0.602
Legumes		
Never	90.75±15.15	140.75±34.98
Rarely	97.47±16.97	148.00±37.69
≥1 time/week	100.15±18.98	147.70±32.48
p value	0.238	0.787
Milk		
≤2 times/week	93.50±14.98	137.59±32.58
3–5 times/week	96.96±15.18	144.59±35.02
6–7 times/week	102.39±20.59	152.61±31.92
p value	0.057	0.084
Eggs		
Never	98.18±20.88	140.62±30.91
1–2 times/week	97.85±17.22	146.52±32.77
≥3 times/week	102.29±18.19	156.42 ±35.42
p value	0.528	0.137
Fruits		
≤2 times/week	94.68±16.41	141.52±30.81
3–5 times/week	99.18±23.17	139.35±24.32
6–7 times/week	100.55±18.23	150.73±35.16
p value	0.324	0.246

(60.6%, κ=0.220, p=0.010), eggs (56.1%, κ=0.337, p<0.001), and fruits (74.2%, κ=0.489, p<0.001). Similarly, the analysis showed a strong agreement between children and fathers in the consumption of red meat/chicken (81.8%, κ=0.664, p<0.001), fish (86.4%, κ=0.766, p<0.001), green vegetables (77.3%, κ=0.581, p<0.001), and legumes(83.4%, κ=0.628, p<0.001), while a moderate or weak one was noted in the consumption of milk (51.6%, κ=0.129, p=0.103), eggs (62.1%, κ=0.411, p<0.001), and fruits (65.1%, κ=0.284, p<0.001). Furthermore, it was revealed that 34.9% and 31.8% of adults were consuming less milk and eggs, respectively, than their children did.

The Cu/Zn ratio among the whole cohort study ranged from 0.77 to 2.69, with a mean value of 1.53±0.44 (median 1.47). There was no statistically significant effect of parents' sex on the Cu/Zn ratio (men 1.48±0.42 vs women 1.59±0.46; *t* test, $p=0.168$). On the other hand, there was a statistically significant negative correlation between parents' age and the Cu/Zn ratio. In this regard, Cu/Zn ratio tended to decrease with increasing age (age< 30 years 1.78±0.45, 30–39 years 1.47±0.38, ≥40 years 1.40±0.45 one-way ANOVA, $p<0.001$). More specifically, the Cu/Zn ratio in adults <30 years old did not statistically significantly differ from the ratio in children (1.76±0.59, $p=0.791$), but it was higher than that in adults 30–39 years old ($p=0.002$) and that in adults ≥40 years old ($p=0.001$). No significant difference was found between the other two age intervals ($p=0.696$).

Discussion

The first aim of this study was to determine the levels of Cu and Zn among a healthy children population and their parents and to identify the relation between these groups' levels. There are two relative studies in Greece: the first one concerning children, aged 3–14 years, living in Thrace [14] and the second one concerning adults, aged 18–60 years, living in a region of Athens [15], but there are no previous reports on both healthy children and their parents.

The mean level of Cu in children was 167.02±35.07 μg/dl, which is higher than those reported by Ahmed et al. [16] for children of urban Bangladesh, by Alarcón et al. [17] for schoolchildren residing in Meridá, Venezuela, by Ohtake and Tamura [18] for healthy Japanese children, and by Yakinci et al. [19] for Turkish children. On the other hand, Zn level in children was 99.88±19.08 μg/dl, which is in close agreement with those determined by Ohtake and Tamura [18] and by Hogberg et al [20], but it was higher than those reported by Alarcón et al. [17] and by Yakinci et al. [19].

The mean level of Cu in adults was 147.10±33.11 μg/dl, which is higher than those defined by Kouremenou et al. [15] for Greek adults, by McMaster et al. [21] for the population of Northern Ireland, and by Rukgauer et al. [22] for German healthy children and blood donors. On the contrary, Zn level was 98.99±18.54 μg/dl, which was in agreement with those mentioned by Rukgauer et al. [22], but it was higher than those reported by Kouremenou et al. [15] for Greek adults, by McMaster et al. [21] for the population of Northern Ireland, and by Gattás et al. [23] for young Chilean adults.

The different serum levels of the trace elements among the several surveys are due to the different cultures, religions, and socioeconomic status of the studied populations, which mainly affect their diet. The country of origin also plays a major role, since the solid has different homogeneity that affects the composition of the agricultural products in bioelements.

In our study, there was no statistically significant overall difference in Zn levels between males and females, either in children or in their parents, also reported by Ohtake and Tamura [18], Rukgauer et al. [22], Diaz Romero et al. [24], Farzin et al. [25], and by Song et al. [26], but it conflicts with the results of Kouremenou et al. [15], McMaster et al. [21], and Gattas Zaror et al. [23]. On the other hand, Cu levels in women were higher than those in men, relation that was also found by other authors [11, 15, 21, 25], while no correlation was noted between children and Cu levels, finding that agrees with those of other authors [18, 22, 26].

No statistically significant correlation was observed between Zn levels and age among parents, which is in accordance with previously reported results in adults [15, 24], whereas a positive association was found between Zn levels and age among children, relation that is in conformity with that observed by Alarcon et al. [17], but it differs from the reports of Ohtake and Tamura [18]. On the contrary, there was a negative correlation between Cu levels and age among both children and their parents, as it has also been shown by other authors [18, 22, 24], but it is opposite to the results of Kouremenou et al. [15] and McMaster et al. [21]. Thus, Cu levels in children were statistically significantly higher than those in parents, whereas there was no significant difference in Zn levels between children and their parents. In addition, a statistically significant negative correlation between Cu/Zn ratio and age was found, as Cu/Zn ratio tended to decrease with age.

But is there an association between Cu and Zn levels in children and those in their parents? We found a significant positive correlation between children's Zn levels and those in their mothers, while children's Cu levels were positively correlated to their both parents' Cu levels. Of course these values could partly be genetically determined, but diet is a factor that can be modified in order to have higher Cu and Zn levels. Therefore, the main purpose of this study was to investigate the relation between children's and parents' nutritional habits, which can influence the levels of these bioelements.

There are many dietary factors that influence the absorption of Zn and Cu, acting either as enhancers or as inhibitors [27, 28]. Therefore, it is necessary to define the relation between Zn and Cu levels and Greeks' nutritional habits. In our previous study in children [14], we found a strong positive correlation between Zn levels and meat, milk, and eggs, whereas Cu levels tended to increase as the consumption of fish, green vegetables, legumes, and fruits was increasing. In this study, we observed a statistically significant positive correlation between parents' Zn levels and meat and milk, while parents' Cu levels increased with the consumption of fish and milk. The different results between the two studies depend on the homogeneity of the diet among children and their parents. As for the correlation between Cu levels and milk, it needs further investigation, since it is well known that milk is poor in Cu.

Children begin to mimic their parents' food choices at a very young age. Therefore, we tried to identify the parental influence on children's nutritional habits that affect the concentration of Zn and Cu in serum. A strong agreement was observed between both parents and children in the consumption of meat, fish, green vegetables, and legumes and a moderate one in the consumption of milk, eggs, and fruits. This small difference depends on parents' aspects of healthy food intake and on the fact that they use more “pressure to eat” these healthy foods on their children [29]. Thus, we noted that parents were consuming less eggs and milk than their children, foods that are considered to be necessary for growth and development.

Moreover, mothers are of particular interest on children's eating behavior [30]. Thus, we noted strong positive correlations between children and their mothers in Zn and Cu levels, while a significant positive correlation between fathers and children was found only in Cu levels.

In conclusion, children’s Cu and Zn levels are related to their parents’ levels. Moreover, there are positive correlations between the serum levels of these trace elements and the nutritional habits. Therefore, parents can influence their children’s nutritional habits, in order to have higher serum Cu and Zn concentrations.

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