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Poor zinc and selenium status in phenylketonuric children and adolescents in Brazil

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Abstract

Because of the restricted intake of high-biologic-value protein, children with phenylketonuria (PKU) may have lower than normal plasma concentrations of copper, zinc, and selenium. The purpose of the present study was to investigate erythrocyte zinc levels and serum copper and selenium levels in children and adolescents with PKU by analyzing the relation between their diet and the laboratory profiles of these elements. The study was conducted in 32 children and adolescents with PKU, who were on a special diet. Dietary records and blood samples were collected from each subject. Erythrocyte zinc and serum selenium levels were below normal in 37.5% and 90.6% of the subjects, respectively. Plasma copper levels were normal. Metabolic formulas were the only source of 86.9% of the zinc, 65.6% of the copper, and 32.4% of the selenium. Despite this, there was no significant correlation between the zinc formula supply and erythrocyte zinc levels ($\rho = -0.143$, $P = .435$) or the supply and serum levels for copper ($\rho = -0.117$, $P = .523$) and selenium ($\rho = 0.113$, $P = .538$). These results suggest that Brazilian patients with PKU present with low ingestion levels, low serum selenium levels, and low erythrocyte zinc levels.

Keywords: Zinc; Selenium; Copper; Dietary therapy; Humans; Phenylketonuria
Abbreviations: DRI, dietary reference intake; PKU, phenylketonuria; RDA, recommended dietary allowance.

1. Introduction

Dietary therapy is the predominant treatment for phenylketonuria (PKU). To maintain the level of phenylalanine within a narrow range, the recommended diet is a low-protein diet that excludes animal products (because of their phenylalanine content) and includes controlled amounts of cereal, fruit, and vegetables, in addition to protein supplementation with phenylalanine-free metabolic formulas [1,2]. The low ingestion of proteins with a high biologic value and the predominance of vegetable-origin foods containing fibers, phytates, oxalates, and tannins in the diet decreases the bioavailability of many nutrients. [3,4]. Some children with PKU have low serum levels of copper and zinc [5-7]. Although some studies report low zinc consumption and low serum zinc levels in children with PKU [6,8], another reported high zinc consumption in children with PKU in comparison with healthy children [4]. Yet, another study [9] demonstrated that selenium values and glutathione peroxidase enzyme activity in the erythrocytes are reduced in children with PKU.

In Brazil, especially the northeastern region of the country, which is characterized by high poverty levels, PKU treatment by dietary therapy is difficult to implement

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because of the small variety of foods available to patients, which could potentially aggravate possible micronutrient deficiencies. Moreover, few studies have assessed the ingestion and biochemical dosage of micronutrients in patients with PKU. Thus, the aim of this study was to investigate the levels of trace elements (zinc, copper, and selenium) in children and adolescents with PKU, by analyzing the relation between the diet consumed and the plasma levels of these elements. We hypothesized that Brazilian patients with PKU present with lower serum levels of trace elements and therefore have a greater dependence on metabolic formulas. The findings of the present study are potentially important for improving nutrition programs for patients with PKU in developing countries.

2. Methods and materials

2.1. Study subjects

This was a cross-sectional study conducted between August 2004 and April 2005, in which 32 patients with PKU (0-20 years of age) were assessed, diagnosed, and followed up by the Reference Neonatal Screening Service of the Parents and Friends of the Handicapped Association–Salvador, Bahia, Brazil. All studies were performed with the approval of the research ethics committee of the Federal University of Bahia. Thirty-two children and adolescents were recruited for the study. The subjects included 18 girls (56.3%) and 14 boys (43.7%) (mean age, 5.02 ± 5.58 years; median, 2.58; range, 1 month to 19 years; 56.3% [n = 18] of the patients were <3 years old, and 6.3% [n = 2] were >18 years old). Twenty-nine patients (90.6%) presented with the classic symptoms of the disease.

2.2. Nutrient intake calculation

Nutrient intake was assessed based on 24-hour and 3-day food records, from which the types and quantities of foods consumed, and the forms of preparation were obtained. All the subjects used the same metabolic formula, with compositions specific for each age group (PKU1 for those under 1 year of age, PKU2 for children between 1 and 8 years of age, and PKU3 for children >8 years of age). Nutrient intake was calculated for each subject separately using the software program Dietwin-Professional (Porto, Alegre, Rio Grande do Sul, Brazil) [10]. The protein-calorie intake and adequacy percentage of nutrients consumed daily by each patient were also calculated based on the specific dietary reference intakes for each age group [11] using the mean consumption between the 3-day food register and the 24-hour record. Proteins, carbohydrates, lipids, phenylalanine, tyrosine, iron, calcium, zinc, copper, and selenium were assessed.

2.3. Blood collection and biochemical analyses

Venous blood (7-10 mL) was obtained from fasting subjects between 7 and 9 AM using EDTA-treated syringes. Samples were placed on ice, protected from light, and centrifuged at 4000*g* at 5°C for 15 minutes. Plasma was collected in airtight vials and stored at −70°C until analyzed. Plasma selenium and copper concentrations were determined using atomic absorption spectrophotometry. The reference range for selenium was 46 to 143 μg/L for all ages, and that for copper was 20 to 40 μg/dL for children 0 to 6 months of age and 70 to 140 μg/dL for children older than 6 months. Erythrocyte zinc concentrations were determined using the reference range of 440 to 860 μg/dL [12,13]. We analyzed erythrocyte zinc concentrations rather than plasma zinc concentrations because they better reflect the chronic deficiency state [14].

2.4. Statistical analysis

Descriptive statistics are expressed as means ± SD. Analyses were performed considering 3 age strata (<1 year, 1 through 8 years, and >8 years), based on the recommendations for metabolic formula use. Continuous variables were expressed as means ± SD. The mean values of the different groups and their SDs were analyzed using the nonparametric Kruskal-Wallis test [15]. Correlation analysis with determination of Spearman correlation coefficient was performed to examine the degree of (linear) relationship between the erythrocyte zinc levels and serum copper and selenium levels and the intake of these nutrients [15]. A P value of .05 or less was considered statistically significant. All analyses were performed using the SPSS statistical package (SPSS, Chicago, Ill) [15].

3. Results and discussion

Mean ingestion of trace elements was: copper 2.2 ± 0.8 mg, zinc 9.9 ± 6.2 mg, and selenium 5.2 ± 1.6 μg. The mean dietary adequacy percentage was: copper 54.7% ± 15.9%, zinc 243.1% ± 146.8%, and selenium 22.8% ± 9.3%. The patients who used the PKU3 metabolic formula had the lowest selenium adequacy levels (P = .003), and those who used PKU1 had the highest zinc adequacy levels (P = .006), although the consumption level of zinc was above the recommended value for all groups (Table 1).

The mean dose values of the trace elements were copper 120.9 ± 18.9 μg/dL, selenium 22.1 ± 16.5 μg/L, and zinc 479.5 ± 139.8 μg/L. Plasma copper levels were normal in all 32 subjects. Plasma selenium levels were within normal limits in 9.4% (3) of the subjects and were below the reference value in 90.6% (29) of the subjects. Erythrocyte zinc levels were normal in 62.5% (20) of the subjects and below the reference value in 37.5% (12) of the subjects.

Despite ingestion far in excess of the dietary reference intake in the study population, erythrocyte zinc levels were below the normal limit in 37.5% of the subjects, which suggests that the bioavailability of this element is altered in PKU patients. The bioavailability of zinc might be influenced by various factors, among them, dietary fiber and phytate content. The phytic acid in grains, legumes, and

Table 1
Mean plasma levels of erythrocyte zinc, copper, and selenium measured in 32 patients with PKU, stratified by type of metabolic formula used

Variable	Type of metabolic formula			
	PKU1	PKU2	PKU3	P^a
Erythrocyte zinc (μg/dL)	435.6 ± 99.1	476.4 ± 122.4	527.2 ± 220.7	.105
Serum copper (μg/dL)	115.0 ± 15.0	125.8 ± 20.5	108.9 ± 6.6	.145
Serum selenium (μg/L)	24.5 ± 14.6	19.5 ± 13.9	29.1 ± 25.4	.504
Zinc from formula in diet (% contribution)	90.8 ± 9.6	86.3 ± 8.8	86.3 ± 11.2	.577
Selenium from formula in diet (% contribution)	51.8 ± 27.4	32.2 ± 15.0	17.2 ± 22.4	.023
Copper from formula in diet (% contribution)	75.8 ± 17.1	62.1 ± 13.8	69.9 ± 16.3	.173
Total zinc consumption (% adequacy)	461.0 ± 190.1	202.2 ± 103.4	204.6 ± 76.1	.006
Total selenium consumption (% adequacy)	29.2 ± 4.3	24.6 ± 8.2	10.9 ± 5.0	.003
Total copper consumption (% adequacy)	65.3 ± 25.6	51.3 ± 11.8	57.9 ± 17.8	.286

Data are expressed as means ± SD for 32 subjects (PKU1 group, n = 5; PKU2 group, n = 21; PKU3 group, n = 6).
[a] Significant differences were determined using the nonparametric Kruskal-Wallis test.

nuts forms insoluble complexes with zinc, leading to an increase in its fecal loss [4,14,16-18].

The plasma copper levels were normal in 100% of the subjects, despite consumption being below the recommended level. In the present study, ingestion below the recommended value did not interfere with the plasma levels of this nutrient. Grooper [4] reported no differences between copper ingestion in healthy children and those with PKU and concluded that ingestion of this nutrient in these patients achieved the daily recommended dietary allowance. Fisberg et al [19] reported no significant differences in the plasma copper levels between a group of children with PKU and the control group. In studies conducted in humans, phytates do not appear to interfere with copper absorption [20,21].

A high percentage of subjects (90.6%) had selenium levels below the reference values. The metabolic formulas used are selenium-free. Acosta et al. [6] in 1987, reported selenium ingestion below 67% of the recommended dietary allowance and a prevalence of plasma selenium levels below the lower limit in 60% of patients with PKU. In another study, Acosta et al [22] reported low plasma selenium levels in 16% of the study population. Reilly et al [23] reported reduced plasma and urinary levels of selenium in children with PKU, and in their study, the selenium ingestion levels were below the recommended value in all the children.

Red meat is the main source of selenium [24]. Grains vary in selenium content based on the richness of selenium in the soils in which they are grown [25]. Selenium plasma levels correspond to alterations related to its ingestion, although glutathione peroxidase activity (a selenium-dependent enzyme) provides a more precise estimate [26].

The copper, selenium, and zinc content values of the prepared metabolic formulas (associated with the carbohydrate, lipid, and milk formula modules) were: copper 1.5 ± 0.7 mg; selenium 1.6 ± 1.1 μg, and zinc 8.6 ± 5.6 mg. The prepared metabolic formula provided 65.7 ± 15.2% of the copper of the total diet, whereas the metabolic formula contributed 32.4 ± 20.7% of the selenium and 87.0 ± 9.2% of the zinc of the total diet.

In this study, patients older than 8 years, consumers of the metabolic formula PKU3, had lower dietary selenium adequacy indexes, which might be because of their greater need for this nutrient, small menu variations, and the patient's difficulty accepting the prepared metabolic formula, frequently preferring to consume the formula with added fruit, which is poor in selenium.

There was no significant correlation between the zinc formula supply and erythrocyte zinc levels (ρ = −0.143, P = .435). The findings were similar for copper (ρ = −0.117, P = .523) and selenium (ρ = 0.113, P = .538), perhaps because of the small number of subjects. There were no correlations between the dosed erythrocyte zinc levels and the total referenced ingestion of calcium (−0.087, P = .636), iron (0.109, P = .554), and copper (0.25, P = .167). Nevertheless, the total zinc ingested in the diet positively correlated with the total ingestion of calcium (r = 0.902, P < .001), copper (r = 0.835; P < .001), and iron (r = 0.863; P < .001), suggesting that chelation might have contributed to the low levels of zinc found in these patients.

In Brazil, the metabolic formulas do not contain selenium. The findings of the present study support our hypothesis that there is high dependence on the formula supply to meet the nutritional demands of Brazilian children with PKU. This may not be the case in other developed countries that offer food supplements that supply micronutrients without phenylalanine.

In conclusion, in Brazilian children, and adolescents with PKU the diet is deficient in selenium, and the bioavailability of zinc may be affected by excess fiber, phytates, and other ingested minerals. These findings will contribute to help developing countries create programs that popularize phenylalanine-free food supplements. These products might be an alternative source of essential micronutrients such as zinc and selenium making it possible to offer these patients better menu options and thus enhance their quality of life.

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References

[1] Mira NV, Marquez UM. Importância do diagnóstico e tratamento da fenilcetonúria. Rev Saude Pública 2000;34:86-96.
[2] Clark BJ. Dietary management for the child phenylketonuria. Eur J Clin Nutr 1992;46(Suppl 1):S532-9.
[3] Acosta PB. Nutrition studies in treated infants and children with phenylketonuria: vitamins, minerals, trace elements. Eur J Pediatr 1996;155(Suppl 1):S136-9.
[4] Gropper SS, Acosta PB, Clarke-Sheehan N, Wenz E, Koch R. Trace element status of children with PKU and normal children. J Am Diet Assoc 1988;88:459-65.
[5] Acosta PB, Fernhoff PM, Warshaw HS, Hambidge KM, Ernest A, McCabe ER, et al. Zinc and copper status of treated children with phenylketonuria. JPEN J Parenter Enteral Nutr 1981;5:406-9.
[6] Acosta PB, Stepnick-Gropper S, Clarke-Sheehan N, Wenz E, Cheng M, Anderson K, et al. Trace elements status of PKU children ingesting an elemental diet. JPEN J Parenter Enteral Nutr 1987;11(3): 287-92.
[7] Taylor CJ, Moore G, Davidson DC. The effect of treatment on zinc, copper and calcium status in children with phenylketonuria. J Inherit Metab Dis 1984;7:160-4.
[8] Longhi R, Rottoli A, Vittorelli A, Zecchini G, Bonabitacola T, Bertassi F, et al. Trace elements nutrition in hyperphenylalaninemic patients. Eur J Pediatr 1987;146:32-7.
[9] Lombeck I, Jochum F, Terwolbeck K. Selenium status in infants and children with phenylketonuria and in maternal phenylketonuria. Eur J Pediatr 1996;155(Suppl 1):S140-4.
[10] Institutes of Medicine Dietary Reference Intakes: applications in dietary assessment. Washington DC: National Academic Press; 2001.
[11] Dietwin software, Release 2.0, 1995-2006. Brazil.
[12] Hermes Pardini Institute. Rio de Janeiro, Brazil. Release 2005; Hermes Pardini Laboratory home page available at: http://hermespardini.com.br.
[13] Iyengar V, Woittiez J. Trace elements in human clinical specimens: evaluation of literature data to identify reference values. Clin Chem 1988;34:474-81.
[14] Toral N, Rhein SO, Cintra IP, Fisberg M. O papel do zinco na infância e adolescência. Pediatria Moderna 2005;41(4):158-68.
[15] SPSS Inc. SPSS user guide, release 9.0. Chicago, IL; 1999.
[16] Hunt JR. Bioavailability of iron, zinc and other trace minerals from vegetarian diets. Am J Clin Nutr 2003;78(Suppl):633s-9s.
[17] Mafra D, Cozzolino SMF. Importância do zinco na nutrição humana. Rev Nutr Campinas 2004;17(1):79-87.
[18] World Health Organization. Vitamin and mineral requirements in human nutrition. Geneva: World Health Organization and Agriculture Organization of the United Nations; 2004.
[19] Fisberg RM, Da Silva-Fernandes ME, Fisberg M, Schmidt BJ. Plasma zinc, copper, and erythrocyte superoxide dismutase in children with phenylketonuria. Nutrition 1999;15:449-52.
[20] Hunt JR, Vanderpool RA. Apparent copper absorption from a vegetarian diet. Am J Clin Nutr 2001;74:803-7.
[21] Lonerdal B. Bioavailability of copper. Am J Clin Nutr 1996;63 (Suppl):821S-9S.
[22] Acosta PB, Yannicelli S, Marriage B, Steiner R, Gaffield B, Arnold G, et al. Protein status of infants with phenylketonuria undergoing nutrition management. J Am Coll Nutr 1999;18(2):102-7.
[23] Reilly C, Barrett JE, Patterson CM, Tinggi U, Latham SL, Marrinan A. Trace element nutrition status and dietary intake of children with phenylketonuria. Am J Clin Nutr 1990;52:159-65.
[24] Pennington JAT, Young BE. Total diet study nutritional elements, 1982-1989. J Am Diet Assoc 1991;91(2):179-83.
[25] Anderson JJB. Minerals. In: Mahan LK, Stump SE, editors. Krause's food, nutrition and diet therapy. 10th ed. Philadelphia: Elsevier; 2002. p. 642-66.
[26] Litov RE, Combs GF. Selenium in pediatric nutrition. Pediatrics 1991;87(3):339-51.