

Plasma Zinc, Copper, and Erythrocyte Superoxide Dismutase in Children With Phenylketonuria

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ABSTRACT

Children with phenylketonuria (PKU) are treated with semisynthetic diets restricted in phenylalanine (PHE). The formulae must supply those trace elements and vitamins that are usually supplied by whole protein foods. We studied the effects of phenylalanine restricted diets in 42 children with PKU (P) and 31 normal (N) children, aged 1–12 y, divided into two groups (below and above 7 y). Plasma zinc and copper were analyzed by means of atomic spectrophotometry, and superoxide dismutase (CuZnSOD) activity was measured in erythrocytes, through NBT inhibition and its profile, as determined by isoelectric focalization. Plasma zinc of PKU children ≥ 7 years old was significantly lower than that in the control group (17 $\mu\text{mol/L}$ versus 20 $\mu\text{mol/L}$) but still within the normal range; in children < 7 years no substantial differences were found between the two groups. Plasma copper was not statistically different between PKU and normal children. Qualitative activity of CuZnSOD presented the same electrophoretic profile in both normal and PKU. Quantitative activity was not different in both P (1210 U/g Hb < 7 versus 1328 U/g hemoglobin (Hb) ≥ 7) and N (1675 U/g Hb < 7 versus 1367 U/g Hb ≥ 7). We concluded that children with PKU presented normal mean levels of zinc and copper, with preserved function, measured by enzyme activity. *Nutrition* 1999; 15:449–452. ©Elsevier Science Inc. 1999

Key words: phenylketonuria, zinc, copper, superoxide dismutase, trace elements, artificial diets

INTRODUCTION

Children with phenylketonuria (PKU) receive semisynthetic formulae restricted in phenylalanine (PHE), since usual protein intake increases PHE excessively.

Treatment using semisynthetic and synthetic food may cause nutritional deficiency, possibly affecting the absorption of trace elements, because the diet is poor in animal protein and lactose and rich in plant fibers and phytates.¹

The intake of phosphorus, magnesium, potassium, copper, and zinc is lower in children with PKU in comparison with normal children, since the sources of these metals (meat and its derivatives and eggs) are completely eliminated from the diet² and these elements must be supplied through artificial formulae.

All these conditions arise in the course of the dietetic treatment of patients with PKU, and numerous cases of Zn, Cu,^{1–4,6} Se,⁵ and

sometimes Fe and/or ferritin³ deficiencies have been noted in these subjects. However, the associated clinical manifestations are not clear. Based on these facts, we evaluated the trace elements zinc and copper through their plasma levels and the enzymatic activity dependent on these metals in children with PKU.

MATERIALS AND METHODS

Subjects

Forty-two phenylketonuric children of both sexes aged 1–12 y were divided into two groups, under ($n = 24$) and over ($n = 18$) 7 y of age, took part in this study and were followed-up at “Associação de Pais e Amigos dos Excepcionais (APAE) de São Paulo” (Association of Parents and Friends of Handicapped Children). The control group consisted of 31 normal children ($n < 7$ y = 9, $n > 7$ y = 22), who attended municipal day-care centers in

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TABLE I.

% RDA INTAKE OF ENERGY, PROTEIN, ZINC, AND COPPER ACCORDING TO AGE				
Group intake (source)	<7 y		≥7 y	
	PKU2 15 g	Dietary	PKU2 25 g	Dietary
Energy	3.0	62.7	4.0	60.5
Protein	63.0	100.9	61.0	109.4
Zinc	12.0	36.6	20.0	38.5
Copper	30.0	85.8	50.0	104.1

São Paulo, Brazil, and presented characteristics similar to those of the above group.

The PKU children were divided into two age groups because the average amount of the special offered product was different for the two age groups, according to the product use instructions given by the manufacturer.

Diet

The formulae used by children with PKU was PKU2 (Milupa, Friedrichsdorf, Germany), phenylalanine-free, with vitamin, mineral salt, and trace element supplementation.

The formulae must be taken according to the manufacturer's instructions (based on body weight) three times a day: children under 7 y take 15 g on the average and those over 7 y take 25 g (Table I). The remaining diet consists of cereal, fruits, vegetables, but no food of animal source. It is important to mention that low-protein foods are not easily available in our country, and the diet is calculated based on nutritional data taking into consideration phenylalanine content and other nutrients. The children are oriented by means of a menu and a replacement list.

Since we estimate the energy and nutrients by means of a 3-d dietary record, the results show the following mean percentage intake of RDA, according to Table I. The results show that it is more difficult for patients to reach the amount of calories recommended than the amount of protein recommended, since the protein recommendation can be reached by intake of the available formulae; however, energy requirements are not met.

The control group remained in day-care centers during the whole day, getting four meals daily, that is, only dinner was at home with these institutionalized and controlled children. These children received 80–90% of their energy and other nutrient intake while in the day-care center. There were no restrictions regarding meals taken at home.

In evaluating the nutritional status of the two groups, there were no great differences regarding weight/height (W/H), weight/age (W/A) indices, nevertheless, height/age (H/A) presented significant differences in children under the age of 7 with PKU. When compared with normal children of the same age, the statural increment was shown to be impaired. In the group with PKU, 11.6% were below the third percentile for height for age when compared to the normal children, where 2.8% were below this value.

Data Collection and Procedures

Five mL of venous blood were drawn using disposable plastic syringes containing 0.1 mL of heparin (100 IU). Half of the blood was transferred to polypropylene tubes and centrifuged for 10 min at 3000 rpm. The plasma was separated and kept in covered polypropylene tubes and stored at -18°C , until plasma zinc and copper were measured. The remaining blood was used to deter-

TABLE II.

PLASMA Zn ($\mu\text{mol/L}$) OF CHILDREN WITH PHENYLKETONURIA (PKU) AND NORMAL (N) CHILDREN, ACCORDING TO AGE			
Group	Mean	Standard deviation	Confidence interval
<7 y			
PKU	15.6	2.6	16.7–14.5
N	16.8*	3.3	19.0–14.6
≥7 y			
PKU	16.8*	3.6	18.5–15.1
N	20.0*	3.9	21.6–18.4

Normal range, 10.0–30.0 $\mu\text{mol/L}$.

* $P \leq 0.05$.

mine superoxide dismutase (SOD) activity. The samples were transferred to polypropylene tubes, and centrifuged at 1000 rpm for 5 min to separate erythrocytes and plasma. Erythrocytes were washed three times with saline (NaCl 0.9%) and centrifuged at 1000 rpm for 5 min. "Pools" of erythrocytes were then obtained and frozen at -18°C , and defrosted three times for cell hemolysis. Precautions against environmental trace metal contamination were taken for specimen collection, handling, and analyses. Distilled, deionized water and ultra high purity reagents were used in sample and reagent preparation. Before use, all glass and plastic utensils were washed with 1.0 N nitric acid and deionized water.

Plasma concentrations of zinc and copper were determined spectrophotometrically (Perkin-Elmer, Model 5100 PC). Superoxide dismutase (SOD) followed the method of Abdalla et al.⁷ The enzymatic profile was determined by isoelectric focusing. We standardized a new method with agarose gel IEF⁸ to study SOD. For the preparation of the gel, 0.3 g of agarose IEF, 3.6 g of sorbitol, and 27 mL of bidistilled water were mixed and heated in a water bath until the agarose was completely dissolved. At 75°C (167°F), 1.9 mL of ampholyte (pH 4.0–6.5) were added. The gel was collected on a $180 \times 130 \times 1$ mm glass plate. For electrophoretic activity, 1 μL of each hemolyzed sample was applied, in a small slot, 2 cm away from the cathode; four Whatmann paper bands (18×1 cm) were dipped into the anode solution (H_2SO_4 10M), and four bands into the cathode solution (NaOH 1M), and placed over the gel, 13 cm away from each other. Focusing was by maintaining 15W constantly at 4°C for 90 min. After focusing, the sides of the gel that were in contact with the electrolytic solutions were discarded. We developed the IEF bands according to the method described by Flohé and Otting.⁹

TABLE III.

PLASMA Cu ($\mu\text{mol/L}$) OF CHILDREN WITH PHENYLKETONURIA (PKU) AND NORMAL (N) CHILDREN, ACCORDING TO AGE			
Group	Mean	Standard deviation	Confidence interval
<7 y			
PKU	19.1	6.6	21.8–16.4
N	19.1	3.4	21.4–16.8
≥7 y			
PKU	17.5	5.8	20.3–14.7
N	18.5	7.3	21.6–15.4

Normal range, 15.0–29.4 $\mu\text{mol/L}$.

TABLE IV.

ENZYMATIC ACTIVITY OF SUPEROXIDE DISMUTASE (U/g Hb) OF CHILDREN WITH PHENYLKETONURIA (PKU) AND NORMAL (N) CHILDREN, ACCORDING TO AGE			
Group	Mean	Standard deviation	Confidence interval
<7 y			
PKU	1209.5	±599.5	1437.9–981.1
N	1674.5	±1037.0	2365.8–983.2
≥7 y			
PKU	1326.9	±608.9	1616.8–1037.0
N	1366.9	±779.6	1698.6–1035.2

No normal range available.

Statistical Analysis

The Mann-Whitney *U* test¹⁰ was used to compare the two groups of children in relation to the studied parameters by approximation to the curve of normal distribution (*z* statistics) when necessary due to sample size.

RESULTS

The amount of plasma zinc found in normal children <7 y old was significantly lower than that in the >7-y-old children (Table II). When children with PKU were compared to normal children, in those <7 y old, the mean plasma zinc was higher than in the normal children, but the difference was not significant. In those >7 y old, the means for phenylketonuric and normal children showed significant differences, 17 $\mu\text{mol/L}$ and 20 $\mu\text{mol/L}$ respectively.

Plasma copper levels (Table III) in the groups of normal children and in those with PKU did not present marked differences and mean values were similar to those found by other authors.¹¹

Table IV shows that there were no significant differences between the means of superoxide dismutase activity in the assessed groups. However, male phenylketonuric children aged <7 y presented a lower SOD mean (851.5 U/g Hb) than normal children of the same age group and sex. According to the qualitative analysis of SOD (Fig. 1) we found no different characteristics in the band patterns of phenylketonuric (P) and normal (N) children.

DISCUSSION

Mean plasma zinc levels were higher in normal children when compared to children with PKU. Longhi et al.,¹ McMurry,⁴ and

Taylor et al.⁶ found similar results, with significantly lower plasma zinc levels in phenylketonuric children than in their controls.

Phenylketonuric children also used as a protein source hydrolysates with a minimum amount of PHE or amino acid mixtures. Gropper et al.¹² evaluated 10 phenylketonuric (4–13.8-y-old) and 9 normal (6.5–15.9-y-old) children whose mean plasma zinc levels did not differ (11.3 and 11.5 $\mu\text{mol/L}$ respectively). In that study, the mean zinc intake was significantly higher than that of the control group. However, plasma concentration of zinc did not differ, suggesting low bioavailability of this nutrient in the diet of children with phenylketonuria. Fisberg et al.¹³ evaluated plasma zinc levels in eutrophic preschool children and found an average value of 18 $\mu\text{mol/L}$. The mean value found in the present study for children of the same age group was 16 $\mu\text{mol/L}$; therefore, lower than the one previously mentioned. In the group of normal children, none presented with a zinc level <11 $\mu\text{mol/L}$, whereas in the group with PKU, 3 children showed concentrations similar or lower than those previously mentioned. The reference value (<11 $\mu\text{mol/L}$) is determined in most of the studies as the lowest value within normal standards in children.¹⁴

Gropper et al.¹² showed that there was no difference between plasma copper concentration of children with PKU (19.2 $\mu\text{mol/L}$) and their control group (18.7 $\mu\text{mol/L}$), finding similar means to those of our study. In a study carried out with 21 phenylketonuric children, aged 1 mo–8 y, significant differences were found between the plasma copper means of these patients (29.5 $\mu\text{mol/L}$) and those of normal children (19 $\mu\text{mol/L}$)¹⁵ unrelated to copper intake.² Although the means are within normal range, in the present study 13% of normal children and 24% of phenylketonuric children showed plasma copper levels lower than 14 $\mu\text{mol/L}$. However, none of them showed serious malnutrition or clinical conditions that could justify this result. It is important to mention that hypocupremia was characterized when values were lower than 14 $\mu\text{mol/L}$, found in studies with Chilean children.¹³ Catwright and Wintrobe¹¹ defined hypocupremia when values were lower than 12.6 $\mu\text{mol/L}$. Therefore, the levels defining hypocupremia in our population may be lower than those already reported and these children do not suffer from hypocupremia. Uauy et al.¹⁶ conducted a study with 19 children suffering from malnutrition whose copper values were lower than 14 $\mu\text{mol/L}$, and 9 of these children received a supplement of copper sulfate (80 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) for 120 d. The levels of plasma copper (15 versus 26 $\mu\text{mol/L}$), ceruloplasmin (330 versus 500 mg/L), and SOD (1073 versus 1371 U/g Hb) rose significantly after supplementation. The authors found mean SOD values after supplementation similar to those of our study. In the literature no

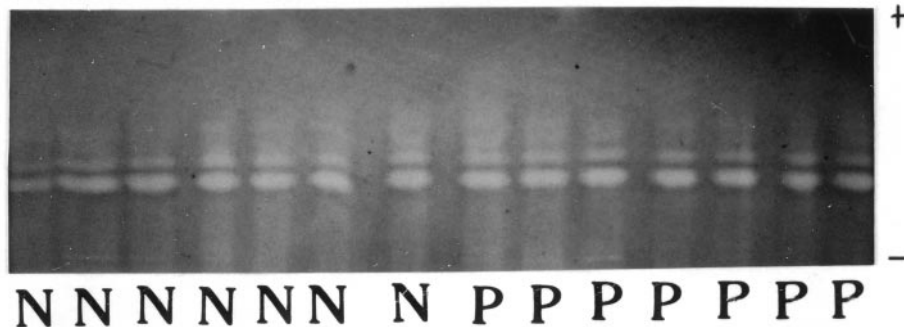


FIG. 1. Profile of superoxide dismutase (SOD) (electrofocusing) of children with phenylketonuria (PKU) and normal (N).

maximum and minimum values of the enzymatic activity for children are defined, making the assessment of individual data difficult.

The qualitative enzyme analysis, done in order to observe its electrophoretic profile, showed the capability of phenylketonuric children to synthesize SOD with the same characteristics of the enzyme synthesized by normal children.

There must be an increase of 400% in the amount of zinc in the formulae, because the amounts of intake by children with PKU were approximately 40% of the recommended amounts, and statistically significant differences were observed regarding plasma level in children of the higher age group (>7 y) as compared to phenylketonuric and normal children. Regarding copper intake, an increase in quantity would not be recommended, since the intake

showed that at least 84% met the recommended requirement, and there were no significant differences between plasma levels in both groups. It should be emphasized that foods that are zinc and copper sources are similar. However, these elements are not present in the same amounts, and diets consisting basically of vegetables (except for Leguminosae) and fruits, such as the diet used in the present study, make it easier to fulfill the recommended copper intake (1 mg) than that of zinc (10 mg).

Although the study as a whole reports mean values of the trace elements zinc and copper within normal standards and preserved function, individual data show values lower than those found in the group of normal children and point out the importance of constant nutritional surveillance and assessment of food intake by these patients.

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ANNOUNCEMENT

The Board of Oxford Nutrition Ltd. is pleased to announce the following appointments to strengthen the management of the company with effect from 1st April 1999.

Professor Gil Hardy is appointed **Chairman**. Gil is also Professor of Pharmaceutical Nutrition in the School of Biological Sciences at Oxford Brookes University and a member of council of The Nutrition Society. He will remain Scientific and Regulatory Consultant to the Company.