Report

Biotin recycling impairment in phenylketonuric children with seborrheic dermatitis

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Abstract

Objective To investigate the effect of a therapeutic diet on serum biotin levels and to explain the seborrheic dermatitis in phenylketonuric (PKU) patients on a "loose" diet.

Design Forty-seven patients were divided into two groups: group A ($n = 21$) demonstrated good compliance to a special diet and group B ($n = 26$) were on a "loose" diet. Most of the patients in group B (20/26), who suffered from mild seborrheic dermatitis, were requested to return to phenylalanine (Phe)-restricted diet for at least 15 days. Seventy-nine healthy children of comparable age were used as controls. Biotin serum levels and plasma biotinidase activity were measured in patients as well as controls. In addition, biotinidase activity was evaluated *in vitro* after incubation with various concentrations of Phe.

Results Biotin levels in group A patients (636 $\pm$ 118 ng/L) were statistically significantly elevated ($P < 0.01$) compared with those of group B patients before (412 $\pm$ 184 ng/L) and after (501 $\pm$ 160 ng/L) 15 days on a Phe-restricted diet, as well as with those of controls (337 $\pm$ 290 ng/L). Furthermore, biotinidase activities were decreased in group B patients (4.2 $\pm$ 1.68 nmol/min/L) compared with those of group A patients (6.4 $\pm$ 0.7 nmol/min/L) and controls (6.10 $\pm$ 0.8 nmol/min/L). Additionally, biotinidase activities in the patients of group B were restored to normal (5.78 $\pm$ 0.81 nmol/min/L), with a simultaneous remission of their skin lesions, after 15 days on a Phe-restricted diet. Moreover, the *in vitro* findings showed a 51% inhibition of biotinidase activity when incubated with Phe (20 mg/dL).

Conclusions It is suggested that the high biotin levels in group A patients reflect the intake of water-soluble biotin of vegetable origin. In contrast, the low biotinidase activity in group B patients may be attributed to their high Phe plasma levels, which acts as an enzyme inhibitor, as shown by the *in vivo* and *in vitro* results. Consequently, the observed seborrheic dermatitis in PKU children (group B) is associated with an impairment of biotin recycling.

Biotin is one of the lesser known water-soluble vitamins of the B-complex group. This compound is directly involved in the important metabolic processes of gluconeogenesis, fatty acid synthesis, and the catabolism of several branch-chain amino acids. It is a prosthetic group in four carboxylase enzymes which occur in human tissues: pyruvate carboxylase, acetyl-CoA carboxylase, propionyl-CoA carboxylase, and β-methylcrotonyl-CoA carboxylase.[1,2] Each of the carboxylases is synthesized as an inactive apoenzyme that is subsequently biotinylated to form holocarboxylase. Humans cannot synthesize biotin, and therefore derive the vitamin from dietary sources (protein-bound and free forms), from the endogenous turnover of carboxylase, and also possibly from the synthetic activity of the gastrointestinal microflora.[3] The enzyme biotinidase plays an essential role in the recycling of endogenous biotin,

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as well as in the processing of protein-bound dietary biotin. Errors in biotin turnover in humans are often manifested by dermatologic problems.[3]

Classical phenylketonuria (McKusick 261600) is currently treated by a special diet given to affected children, so as to avoid an elevated blood concentration of the amino acid phenylalanine (Phe). The diet must be nutritionally adequate in all respects. The recommended intake of nutrients is similar to that of other children, except for Phe and tyrosine.[3,4] In other words, there must be a large reduction in animal products (meat, milk, eggs) in the diet and an increase in the intake of foodstuffs of vegetable origin (vegetarian diet).[4,5] Phenylketonuric (PKU) patients who do not strictly adhere to the above special diet often exhibit dermatologic problems In addition, according to our preliminary data, biotinidase activity was found to be normal in PKU patients with good compliance to the special diet, but was decreased in PKU patients who did not adhere well to this treatment.[6,7]

In an attempt to explain the seborrheic dermatitis in PKU patients on a "loose" diet, we determined the serum biotin levels as well as the effect of the Phe concentration in biotinidase activity.

Patients and methods

Patients

Forty-seven PKU patients were characterized by a screening method,[8] and were placed on a special diet after a tetrahydrobiopterin (BH_4, obtained from Milupa AG) loading test and dehydropteridine reductase (DHPR) evaluation. Their protein intake was largely replaced by PKU_2-Milupa which is a Phe-free mixture of amino acids. This product contains 4.5 μg of biotin per 100 g of powder. The PKU patients were further classified into two groups according to their annual mean plasma Phe (Phe mean) concentrations. Group A consisted of 21 patients (mean age 4.78 ± 3.51 years; Phe mean 2.0 ± 0.9 mg/dL) who showed good compliance with the special diet. Group B included 26 patients (mean age 7.87 ± 3.68 years; Phe mean 15.87 ± 5.09 mg/dL) who did not strictly adhere to the special diet. Most of them (20/26) suffered from mild generalized seborrheic dermatitis. No evidence for the presence of *Pityrosporum* was observed in their dermatologic lesions. As a control group, 79 apparently healthy children, matched for age (mean age 6.68 ± 2.3 years) and sex, were used in this study. The daily nutritional intake of each child was evaluated by a 1-week dietary protocol written by the mother, in combination with a final 24-h dietary recall interview made by the doctor. The amounts of daily nutrients were then calculated according to a coded food list.[4]

Five milliliters of blood was drawn from each group member for biotin, biotinidase, and Phe evaluation (Phe Inst) at the start of the study. Furthermore, all group B members were requested to return to a restricted low-Phe diet for at least 15 days (group B + D). Blood (5.0 mL) was drawn for Phe, biotin, and biotinidase re-estimation at the end of this time. The PKU patients and the control group had no clinical or laboratory evidence of hepatic disease.

The study was carried out with the permission of the Greek Ethical Committee.

Phenylalanine assay

Two to three drops of blood were placed on a Guthrie card for Phe Inst estimation. The concentration of Phe was measured by a quantitative enzymatic assay on dried blood samples spotted on filter papers 2992 Schleicher and Schull with Quantase TM Diagnostic reagents.[8] The Phe mean concentration obtained by assaying a 6-mm dried blood spot was 1.2 ± 0.5 mg/dL for the controls.

Biotin assay

An enzyme-linked method was used to determine serum biotin levels.[9] Briefly, the samples to be assayed were incubated in polystyrene test-tubes with streptavidin-conjugated horseradish peroxidase (Sigma Chemical Co., St Louis, MO). Aliquots were transferred into microtiter plate wells (Costar, Cambridge, MA) precoated with biotinylated bovine γ-globulins. After incubation, the plate was washed and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS, obtained from Sigma Chemical Co., St Louis, MO) and H_2O_2 were added. Color development was read at 405 nm, with a 490-nm reference filter.

Biotinidase assay

The fluorometric assay of biotinidase was carried out using a Perkin-Elmer model LS3 fluorometer according to the method described by Ebrahim and Dakshinamurti.[10] In addition, the effect of Phe (Serva) on a known plasma biotinidase activity was evaluated in triplicate after incubation at 37°C for 30 min with various concentrations (2, 5, 10, 15, 20, 25, 30 mg/dL) of the amino acid.

Statistical analyses

Student's *t*-test was utilized for the statistical analysis of the different nutrients; *t*-test analysis for independent values was used for the comparison of biotin concentrations, with $P < 0.05$ accepted as the level of significance. Linear regression analysis was employed for the correlation between the mean biotinidase activity and Phe mean or Phe Inst concentrations, and Spearman test for the correlation between serum biotin levels and biotinidase activity.

Results

As shown in Table 1, energy, protein intake, and biotin from PKU_2-Milupa did not differ between the groups of

Table 1 Estimated 24-h nutrient intakes (mean ± SD) for the two groups of PKU patients and controls

Nutrient	Nutrient intakes (mean ± SD)			Significance of differences (*P*)		
	Group A (*n* = 21)	Group B (*n* = 26)	Control (*n* = 79)	A vs. control	B vs. control	A vs. B
Energy (kcal)	2114 ± 463	2050 ± 487	2078 ± 473	–	–	–
Protein (g)	68.1 ± 12.9	70.9 ± 19.7	72.3 ± 16.9	–	–	–
Natural protein (g)	26.0 ± 7.2	35.0 ± 8.2	–	–	–	0.05
PKU_2 protein (g)	42.0 ± 5.0	35.0 ± 11.5	–	–	–	0.03
Carbohydrate (g)	260 ± 65	222 ± 32	230 ± 56	0.09	–	0.05
Fiber (g)	31.0 ± 7.1	20.9 ± 8.8	12.6 ± 3.4	0.001	–	0.001
Total fat (g)	79.3 ± 25.1	87.4 ± 4.0	98.8 ± 24.4	0.01	–	–
Cholesterol (mg)	318 ± 136	331 ± 123	355 ± 111	0.0001	–	–
PKU_2 biotin (μg)	2.9 ± 8.0	2.71 ± 1.2	–	–	–	–
Total biotin (μg)	168.0 ± 8.0	160 ± 7.0	162.0 ± 9.0	–	–	–

Student's *t*-test was employed for nutrient analysis.

Table 2 Values of parameters determined in the groups

Groups	Age (years)	Biotin*,‡ (ng/L)	Biotinidase activity†,‡ (nmol/min/L)	Phe mean† (mg/dL)	Phe Inst† (mg/dL)
A (*n* = 21)	4.78 ± 3.51	636 ± 118	6.40 ± 0.77	2.10 ± 0.09	3.10 ± 0.9
B (*n* = 26)	7.87 ± 3.68	411.9 ± 184.9	4.20 ± 0.58	15.87 ± 5.09	18.87 ± 3.09
B + D	7.87 ± 3.68	501 ± 160	5.78 ± 0.81	15.87 ± 5.09	3.5 ± 1.0
Controls (*n* = 79)	6.68 ± 2.3	336.6 ± 290.6	6.10 ± 0.80	–	1.2 ± 0.5

*t-test analysis for independent values was utilized for biotin concentration comparisons. $P < 0.05$ was considered to be statistically significant.
†Linear regression analysis was employed to correlate the biotinidase activity and Phe concentrations.
‡Spearman test was used to correlate the biotin concentration and biotinidase activity.

patients. In contrast, protein from PKU_2, fiber (vegetarian diet), total fat, and cholesterol intake between group A patients and the healthy controls differed significantly. In addition, the mean serum biotin levels were significantly higher in group A compared with group B and the healthy controls. The mean biotin level of group B did not significantly differ compared with that of the healthy controls ($P = 0.211$) (Table 2). Furthermore, the mean biotinidase activity in group A was not significantly different compared with that in the healthy controls. The mean activity of the enzyme in group B was significantly lower than that in group A ($P = 0.006$), as well as in the healthy controls ($P < 0.001$). This was restored to normal after 15 days of a low-Phe diet. No correlation was observed between the serum biotin concentrations and biotinidase activity in all the groups ($r = 0.08$). Moreover, the biotinidase activity in group A showed no correlation with either Phe mean or Phe Inst concentrations. In contrast, in group B, there was a negative correlation observed between the enzyme activity and Phe mean ($r = 0.59$) and Phe Inst ($r = 0.60$) concentrations.

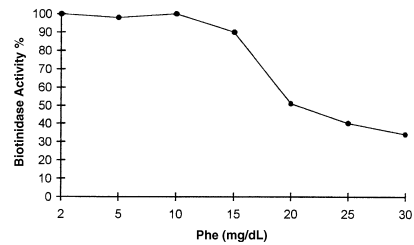

Figure 1 Inhibition of biotinidase by Phe *in vitro*

As shown in Fig. 1, biotinidase activity was inhibited (−51%) when incubated with Phe (20 mg/dL) at 37°C for 30 min.

Discussion

A large percentage of PKU patients, who do not strictly adhere to a special diet and therefore have elevated plasma Phe concentrations, usually exhibit dermatologic problems. As abnormalities in biotin turnover in humans have been associated with dermatologic symptoms,[11,12] we determined the serum biotin levels in 26 PKU patients, 20 of whom (76.8%) presented mild generalized seborrheic dermatitis (group B). We also determined the activity of biotinidase, the enzyme responsible for the recycling of endogenous biotin, which also plays an important role in the processing of protein-bound dietary biotin, in the plasma of these patients. For comparison, the above biochemical parameters were determined in PKU patients who were maintained under a strict diet and had almost normal plasma Phe concentrations (group A), as well as in apparently healthy children of the same average age (group C). A re-evaluation of the above biochemical parameters was performed in patients of group B (group B + D) in whom remission of seborrheic dermatitis was observed after a 15-day, low-Phe diet.

According to our previous results, PKU patients with elevated plasma Phe levels showed a decreased biotinidase activity, which may explain the occurrence of dermatologic problems. Serum biotin levels, evaluated for the first time in PKU patients (group B), did not differ compared with those determined in the control group. PKU patients with normal plasma Phe levels showed normal biotinidase activity, as expected, but their serum biotin levels were higher than those of the control group. This may be explained, at least to some extent, by their strict diet which is based mainly on vegetables. Biotin uptake in humans has not been well studied, and the exact mechanism involved has not yet been completely elucidated. Vegetables and fruit, which represent the main part of the diet of PKU children in group A, contain water-soluble forms of biotin, which are easily absorbed and transported in the bloodstream, as previously suggested,[13] and supported by the present results.

Other mechanisms, yet unknown, may also contribute to the high serum biotin levels. Serum biotin levels did not correlate with the biotinidase activity in any of the groups studied. These findings are in agreement with those of Wolf *et al*.[6] who reported that biotin deficiency did not alter biotinidase activity *in vitro*, and that the activities of the enzyme in the sera of several patients who were biotin deficient were normal.

A number of studies have reported the rapid clearing of seborrheic dermatitis symptoms with biotin treatment in several non-PKU patients;[14,15] however, in most of these studies, neither serum biotin levels nor biotinidase activity were measured.[16] According to our findings, the dermatologic problems often observed in PKU patients with elevated Phe levels were reflected by an altered plasma biotinidase activity, but not by serum biotin levels. Furthermore, these *in vitro* findings support the suggestion made elsewhere[7] that increased plasma Phe may influence biotinidase activity, acting as an enzyme inhibitor. Finally, it is expected that biotin supplementation in PKU patients with seborrheic dermatitis will have no beneficial effect. In contrast, their skin lesions can be treated by strict adherence to a special low-Phe diet.

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