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Impact of Lipophilic Antioxidants and Level of Antibodies Against Oxidized Low-Density Lipoprotein in Polish Children with Phenylketonuria

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Abstract

The treatment of phenylketonuria (PKU) patients constitutes a phenylalanine (Phe) intake restriction in their diet, which is achieved by adding a special Phe-free amino acid mixture to the diet. It has been reported that this diet could have some micronutrient deficiency. Several authors have also reported an increased oxidative stress or impaired antioxidant status in human and experimental PKU. Our project assessed the concentrations of retinol, alpha-tocopherol, coenzyme Q10, and anti-oxidized low-density lipoprotein (ox-LDL) antibodies in PKU children's plasma. It was found that retinol concentration in PKU children remains within the norm despite a low intake. The lower plasma alpha-tocopherol concentration in PKU children compared with normal children was associated with the lower level of antibodies against ox-LDL. This raises the question whether higher than observed circulatory alpha-tocopherol is indeed beneficial to lower plasma ox-LDL levels. Further studies are needed to explain the genetic factor in PKU patients (*e.g.*, CD36/FAT polymorphism gene). The open clinical question is whether daily supplementation of alpha-tocopherol changes the PKU patients' level of antibodies against ox-LDL. Antioxid. Redox Signal. 16, 179–182.

Introduction

Phenylketonuria (PKU) (OMIM 261600) is a congenital metabolic disease of the autosomal recessive hereditary type. It is caused by a deficiency of the liver enzyme phenylalanine hydroxylase (EC 1.14.16.1) and results in an increased level of phenylalanine (Phe) in the blood. The incidence of PKU in Poland, like in other Central European countries, is 1 in 7000 to 1 in 8000 at birth. At present the number of patients treated for PKU in Poland, as recorded in the national PKU register, is 2020. Polish standards of detecting and treating PKU are comparable to those accepted worldwide (1).

The treatment of PKU constitutes in restricting the Phe intake; this is achieved by replacing the sources of proteins in the patients' diet with a special Phe-free amino acid mixture enriched with micronutrients. It has, however, been reported that this diet specified for PKU patients could have some

Innovation

This study was performed on a large, uniform group of PKU pediatric patients with Phe level within the normal range. The product intake in PKU diet was related to blood concentration of antioxidant vitamins. Retinol, alpha tocopherol, and coenzyme Q10 levels were determined with the authors' own method (5), wherein tests were performed simultaneously in small amounts of blood (250 μl). An interesting correlation was found between lipophilic antioxidants and level of antibodies against ox-LDL in PKU children.

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micronutrient deficiency because of its impaired bioavailability or poor dietary compliance on the part of the patients (1).

Several authors have reported an increased oxidative stress or impaired antioxidant status in human and experimental PKU (2). Other studies have shown that lipophilic antioxidants (such as retinol, alpha-tocopherol, and coenzyme Q10) could be crucial in preventing or slowing down neurogenerative diseases and atherosclerotic process by inhibiting the fatty acid oxidation (4).

Our research project focused on finding the plasma concentrations of retinol, alpha-tocopherol, and coenzyme Q10 in PKU children in Poland. The increase in the lipid oxidization process was assessed on the basis of the determined concentrations of anti-oxidized low-density lipoprotein (ox-LDL) antibodies. The intake of vitamins A and E in the children's low-Phe diet was also determined.

The results of our studies demonstrated that the intake of retinol in the PKU group was lower than in the controls, whereas the intake of vitamin A precursor (beta carotene) was statistically higher. Despite the lower vitamin A intake, the plasma concentration in the PKU children remains within norm. According to current nutrition standards, the PKU diet restricts animal products, the main source of retinol, and plant-based products rich in beta-carotene dominate. This results in the statistically lower intake of retinol by the PKU patients. The children who do not follow nutrition guidelines and consume products rich in retinol have increased blood Phe levels. This is confirmed by the correlation observed between Phe concentration and the intake of retinol. The PKU children's significantly higher carotene intake is a factor that counterbalances a relative retinol deficiency in their diet. This explains why we found plasma levels of vitamin A within norm in PKU patients. The lack of correlation found between retinol and carotene intake and vitamin A plasma concentration may also be due to that.

Vitamin E plays a crucial role in preventing lipid peroxidation. In the PKU group, the average plasma alpha-tocopherol concentration was lower than in the healthy group. A slightly lower intake of this vitamin in the PKU group was also demonstrated when compared with the control, but the difference was not statistically significant.

Available evidence on antioxidant vitamin concentrations and retinol and alpha-tocopherol intakes for PKU patients is not unequivocal. The studies concerned patients of different ages and different levels of metabolic compensation and had been performed in smaller populations than our group. According to Colome *et al.*, the average alpha-tocopherol and retinol concentrations in PKU patients were not found to be statistically different from those of the controls, but coenzyme Q10 concentrations were lower compared with the control rates. The same study demonstrated that despite the lack of differences in the mean alpha-tocopherol concentrations, in 17% of PKU patients the concentration of this vitamin was below the normal level. The study did not assess the intake of retinol and alpha-tocopherol in the control group (4). Schulpis *et al.* (8) demonstrated that the intake of retinol, beta-carotene, and alpha-tocopherol was higher in a group of 22 children with well-controlled PKU than in a group of children with poorly controlled PKU and in the group of healthy children. Plasma concentrations of beta-carotene and alpha-tocopherol were higher for the first group than for the second or the control group. Only the higher PKU children's intake of beta-carotene obtained in the study corresponds with our findings. In the project we did, no differences were found between the studied groups concerning plasma coenzyme Q10 levels. In the study by Colome *et al.* (3), conducted with 25 PKU patients aged 8 to 36, CoQ10 concentration in lymphocytes was found to be lower than that in the healthy group. Studies performed on an animal model demonstrated an inhibiting influence of Phe on CoQ10 synthesis. In our study, involving a children population, no correlation was found between concentrations of coenzyme Q10 and Phe; however, to be included in the study, blood levels of Phe should not exceed the recommended values.

Oxidative stress, confirmed by high anti-ox-LDL antibody titers, plays an important part in the pathogenesis of numerous diseases. In our study, the anti-ox-LDL antibody titers were found to be lower for the PKU children than for the healthy ones. Lower antibody levels probably resulted from a better lipid protection against oxidative modification. It is, however, impossible to rule out that our PKU patients' lower alpha-tocopherol plasma concentration—with no differences concerning this vitamin's intake—results from its use in oxidoreductive processes in lipid membranes. A lower anti-ox-LDL titer seems to confirm this point. However, further studies are necessary to assess that correlation.

Colome *et al.* (4) proved that lipid peroxidation in PKU patients was increased. Literature suggests that it is antioxidant vitamins and not enzymes that provide PKU patients with protection against free radicals. Artuch *et al.* (2) discovered no differences in the activity of erythrocyte antioxidant enzymes such as superoxide dismutase, glutathione reductase, and catalase between PKU children and healthy ones. Schulpis *et al.* (9) showed that the total antioxidant status was higher for patients with well-controlled PKU and the control group than for the patients with poorly treated PKU. The concentration of DNA damage marker, 8-hydroxy-2-deoxyguanosine, was also analyzed and discovered to be higher in the group of patients with badly controlled PKU. However, it remains an open question whether these are primary damages caused by increased Phe levels or an inefficient antioxidant system.

Conclusions and Future Directions

The concentration of retinol in PKU children remains within the normal limit, despite its intake by these children is low. The lower plasma alpha-tocopherol concentration in these children was associated with decreased level of antibodies against ox-LDL. This questions whether higher than observed circulatory alpha-tocopherol is indeed beneficial to lower plasma ox-LDL levels. Familial isolated vitamin E deficiency is also an issue (6). Further studies are needed to explain the genetic factor in PKU patients that contributes to this problem (*e.g.*, CD36/FAT polymorphism gene) (7). The open clinical question is whether daily supplementation of alpha-tocopherol changes the PKU patients' level of antibodies against ox-LDL.

Notes

Study population

The tested group comprised 107 children (46 girls and 61 boys) in the age range of 5–12 years (mean, 8.8; standard deviation [SD], 2.06), with PKU detected by newborn screening. The children were patients of seven Metabolic Clinics at teaching hospitals in Poland. Table 1 shows the selection criteria of the patients for the study.

Table 1. Selection Criteria for the Studied Group

Inclusion criteria	*Exclusion criteria*
Phenylketonuria detected by routine newborn screening.	Medical history that includes chronic diseases:
Following a low-phenylalanine diet from early infancy, according to the standards developed by the National Institute of Mother and Child in Warsaw; keeping phenylalanine levels in the blood within the recommended values.	Glomerulonephritis Chronic diseases of biliary ducts and the liver with and without cholestasis Diabetes Lupus erythematosus
Age between 5 and 12 years.	An infectious illness on the day of qualifying for the study and 3 weeks prior.
Permission of the child's parents or guardians for participating in the study.	Being treated with orally administered glucocorticosteroids.

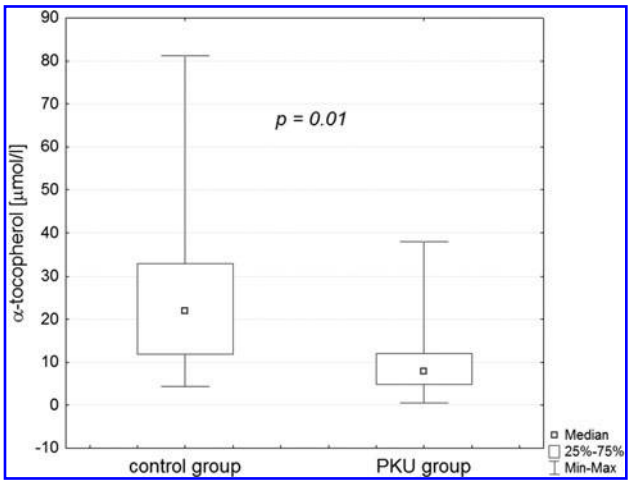

FIG. 1. Plasma alpha-tocopherol concentrations for the children included in the study.

Nutrition information on 107 PKU children was analyzed; for 99 patients, plasma concentrations of vitamins A, alpha tocopherol, coenzyme Q10, and anti-ox-LDL antibodies were measured.

The control group consisted of 62 healthy children (31 girls and 31 boys) aged 5–12 years (mean, 8.6; SD, 1.1) with no history of dietary treatment. All the children from that group had biochemical tests, and a subgroup of 19 randomly chosen children had their diet assessed. The tests had the approval of the Bioethics Committee of the Medical University of Bialystok. The parents of the children included in the study had given their informed consent.

Methods

Blood samples were taken in the morning, at least 12 h after eating. The 1.8 ml samples for measuring the antioxidant vitamin concentrations were collected into test tubes with 3.8% sodium citrate in the ratio 9:1. After a 30-min spin at 4°C, the supernatant was transferred to deep freeze at −70°C. To measure the anti-ox-LDL antibodies, 2 ml of the sample was collected into glass test tubes without the addition of the anticoagulant. After a spin, the serum was stored in a deep freeze at −70°C until measurements were performed. The concentrations of anti-ox-LDL antibodies were determined through an immunoenzymatic method, using Biomedica reagents. The concentrations of retinol, alpha-tocopherol, and coenzyme Q10 were measured simultaneously in one plasma sample with high-performance liquid chromatography (Thermo Separation) (5).

We used a quantitative colorimetric test for measuring blood Phe levels. Phe levels were monitored once a month and blood samples were taken simultaneously. The supply of antioxidant vitamins in the patients' diet was assessed with a 3-day nutrition questionnaire. The information obtained from the questionnaire was analyzed using the DIETA PKU computer program. The program, developed by the members of the research group, includes information on the dietary content of individual nutrients in the food products.

The obtained results were subjected to a statistical analysis, in which the arithmetic mean, median, and SD were calculated for quantitative variables. To determine the characteristics of the normal distribution, the *t*-test was used for independent trials in comparisons between groups. Mann–Whitney *U* test was employed to find the characteristics at variance with such distribution. The Spearman's method was applied in assessing the correlations between changeable characteristics. In these calculations, the relevance level of $p < 0.05$ was accepted as statistically significant. The data were developed with the Statistica 5.0 statistics package.

Table 2. Titers of Antibodies Against Oxidatively Modified Low-Density Lipoprotein Proteins and Anti-Oxidative Vitamin Levels for Groups of Examined Children

	PKU group						*Control group*						
Parameters examined	n	ẍ	*CI −95%*	*CI +95%*	*Median*	*SD*	n	ẍ	*CI −95%*	*CI +95%*	*Median*	*SD*	P
Retinol (*µM*)	99	3.36	2.94	3.79	2.67	2.11	62	2.74	2.21	3.27	2.53	1.93	0.07
α-tocopherol (*µM*)	99	9.89	8.35	11.43	7.91	7.75	62	25.40	20.57	30.23	22.03	17.52	0.01
Q10 coenzyme (*µM*)	99	0.55	0.33	0.77	0.13	1.09	62	0.41	0.24	0.58	0.18	0.62	0.16
Level of antibodies against ox-LDL (mIU/ml)	99	871.64	725.87	1017.41	610.12	703.88	62	1348.71	1120.04	1577.37	1449.85	861.80	0.01

PKU, phenylketonuria; SD, standard deviation; ox-LDL, oxidized low-density lipoprotein.

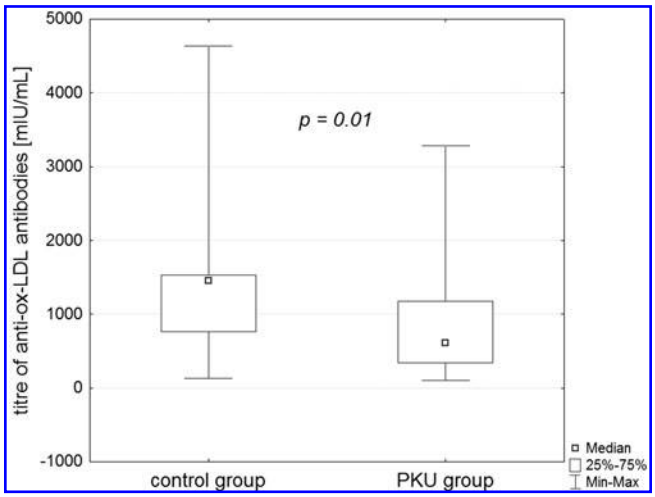

FIG. 2. Serum anti-oxidized low-density lipoprotein antibody titers for the children included in the study.

Results

The tests demonstrated that in the PKU children following a low-Phe diet, the mean value of retinol plasma concentrations was 3.36 μM (SD, 2.11), comparable to the healthy group (2.74 μM; SD, 1.93; $p = 0.07$, bordering on significance) (Table 2). There was a statistically significant difference in the intake of retinol, which was lower in the PKU group ($p = 0.01$)—with a mean of 235.44 μg/24 h (SD, 155.43)—than in the controls (on average, 359.77 μg/24 h; SD, 148.30). Also, a higher intake of vitamin A precursor—beta-carotene—was discovered in the PKU group ($p = 0.02$) [5036.17 μg/24 h (SD, 4065.19) *versus* 2925.11 μg/24 h (SD, 3000.73)].

The studies concerning vitamin E demonstrated statistically lower alpha-tocopherol concentrations ($p = 0.01$) in the PKU children's plasma—9.89 μM (SD, 7.75)—than in the control group (mean value, 25.40 μM; SD, 17.52) (Table 2; Fig. 1). The difference in vitamin E intake by the PKU children and the healthy group borders on statistical significance ($p = 0.07$) and points to a slightly higher intake in the healthy group [7.79 mg/24 h (SD, 5.39) *versus* 8.84 mg/24 h (SD, 3.76)].

The concentration of coenzyme Q10 was 0.55 μM (SD, 0.13) in the PKU children's plasma and 0.41 μM (SD, 0.62) in the healthy group, so the difference was not statistically significant.

We discovered that the anti-ox-LDL antibody titers were statistically lower ($p < 0.01$) in the PKU group [mean value, 871.64 mIU/ml (SD, 703.9)], than in the control group [1348.71 mIU/ml (SD, 861.80)] (Table 2; Fig. 2).

The comparison of the studied parameters in the PKU group showed a relation between the concentrations of Phe and retinol ($R = 0.202$; $p < 0.01$). No correlation was found between the other studied parameters in this group of patients.

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Disclaimer

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Abbreviations Used

ox-LDL = oxidized low-density lipoprotein
Phe = phenylalanine
PKU = phenylketonuria
SD = standard deviation