



## Lipophilic antioxidants in patients with phenylketonuria<sup>1-3</sup>

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### ABSTRACT

**Background:** Low serum ubiquinone-10 concentrations have been described in phenylketonuric patients fed natural-protein-restricted diets. Such low concentrations may be related to increased free radical damage.

**Objective:** We evaluated the relation between low serum ubiquinone-10 concentrations and other lipophilic antioxidants (tocopherol and retinol), selenium, glutathione peroxidase activity, and malondialdehyde concentrations as a marker of lipid peroxidation.

**Design:** This was a cross-sectional study of 58 patients with phenylketonuria (aged 2–36 y; median: 13 y) under dietary treatment, 58 age-matched control subjects, and 30 children with moderate hyperphenylalaninemia fed unrestricted diets (aged 3–17 y; median: 7.5 y). Serum ubiquinone-10 concentrations were analyzed by HPLC with electrochemical detection. Serum retinol, serum tocopherol, and plasma malondialdehyde were analyzed by HPLC with ultraviolet detection.

**Results:** A significant positive correlation was observed between ubiquinone-10 and tocopherol ( $r = 0.510$ ,  $P < 0.001$ ) in the patients with phenylketonuria. After the patients were stratified into 2 groups according to ubiquinone-10 values, significantly lower concentrations of tocopherol were observed in group 1 (low ubiquinone values) than in group 2 (normal ubiquinone values), the hyperphenylalaninemic children, and the control group. Plasma malondialdehyde concentrations were significantly higher in group 1 than in the other groups. No significant differences between groups 1 and 2 were observed in daily intakes of selenium, ascorbate, tocopherol, or retinol.

**Conclusions:** Plasma lipid peroxidation seems to be increased in phenylketonuria. Low concentrations of ubiquinone-10 could be associated with either excessive tocopherol consumption or high malondialdehyde concentrations in patients with phenylketonuria. *Am J Clin Nutr* 2003;77:185–8.

**KEY WORDS** Phenylketonuria, tocopherol, ubiquinone-10, lipid peroxidation, malondialdehyde, oxidative stress, lipophilic antioxidants

### INTRODUCTION

Phenylketonuria (PKU; McKusick 261600) is an inborn error of phenylalanine metabolism resulting from deficient activity of L-phenylalanine-4-monooxygenase (EC 1.14.16.1), the enzyme that catalyzes the synthesis of tyrosine from phenylalanine (1). Phenylalanine accumulation in plasma and tissues as the result of decreased tyrosine biosynthesis seems to be involved in the

pathogenesis of PKU. Reducing the accumulation of phenylalanine through dietary means prevents developmental and mental impairment.

Treatment of patients with PKU consists of restriction of phenylalanine intake, achieved with natural-protein-restricted diets supplemented with a phenylalanine-free amino acid mixture enriched with essential micronutrients, such as vitamins, minerals, and trace elements (2). Despite this supplementation, however, some micronutrients have been found to be deficient in various studies of patients with PKU, probably because of poor dietary compliance with the enriched formulas or impaired bioavailability of some micronutrients (2, 3).

Altered antioxidant status has been reported in PKU, especially in association with selenium deficiency, involving a reduced activity of glutathione peroxidase (GPX; EC 1.11.1.9) (4, 5). Selenium is an essential cofactor for GPX activity, which reflects selenium status (6). Moreover, an increase in plasma lipid peroxidation evaluated as malondialdehyde related to GPX deficiency has been reported in PKU (6). Selenium supplementation is usually applied to prevent selenium deficiency in affected patients, although this supplementation may be insufficient to correct this deficiency (5, 7).

Low serum ubiquinone-10 ( $Q_{10}$ ) concentrations have also been described in patients with PKU consuming natural-protein-restricted diets (8). These low values were mainly associated with high plasma phenylalanine concentrations and, to a lesser extent, low  $Q_{10}$  intakes with the restricted diet (9). Moreover, an inhibition of the rate-limiting enzyme of cholesterol synthesis, 3-hydroxy-3-methylglutaryl CoA reductase (EC 1.1.1.98; common to  $Q_{10}$  biosynthesis) has been associated with high phenylalanine concentrations in experimental hyperphenylalaninemia (10, 11).  $Q_{10}$ , a lipophilic antioxidant that prevents lipid peroxidation in blood and tissues, acts by limiting the formation of lipid peroxy radicals and by reducing other natural antioxidant compounds, such as tocopheroxyl to tocopherol. Therefore, low  $Q_{10}$  concentrations

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**TABLE 1**

Plasma phenylalanine (Phe), ubiquinone-10 (Q<sub>10</sub>), tocopherol, retinol, ascorbate, selenium, and malondialdehyde (MDA) concentrations; glutathione peroxidase (GPX) activity; and daily intakes of tocopherol, retinol, ascorbate, and selenium in patients with phenylketonuria (PKU), children with moderate hyperphenylalaninemia (HPA), and a control group<sup>1</sup>

	PKU patients			HPA group (n = 30)	Control group (n = 58)
	All (n = 58)	Group 1 (n = 25)	Group 2 (n = 33)		
Phe (μmol/L)	604 (152–1407) <sup>2,3</sup>	607 (210–1244) <sup>2,3</sup>	601 (152–1407)	255 (108–607)	50 (33–76)
Q <sub>10</sub> (μmol/L)	0.55 (0.2–1.67) <sup>2,4</sup>	0.37 (0.20–0.45) <sup>2,3,5</sup>	0.57 (0.46–1.67)	0.63 (0.44–1.22)	0.71 (0.36–1.10)
Tocopherol (μmol/L)	20.2 (8.5–30.1)	17.8 (8.5–25.7) <sup>6,8</sup>	21(13.0–30.1)	21.7 (15.1–30.0)	22.0 (12.0–36.0)
Retinol (μmol/L)	1.48 (0.6–3.6)	1.46 (0.6–3.1)	1.55 (0.8–3.6)	1.15 (0.8–1.8)	1.40 (0.6–2.3)
Ascorbate (μmol/L)	70 (36–109)	61 (44–97)	76 (36–109)	67 (38–89)	55 (8–92)
Selenium (μg/L)	49 (11.0–81.0) <sup>2,9</sup>	45 (11.0–68) <sup>2,9</sup>	49 (25–81)	60.6 (28.0–84.0)	65.4 (32.–84)
GPX (U/g Hb)	19.8 (11.4–30.1)	20 (12–30.1)	19.7 (11.4–27.8)	20.4 (14.4–26.0)	21.8 (13.9–33.6)
MDA (nmol/L)	663 (316–1404) <sup>7</sup>	768 (497–1341) <sup>4,7,10</sup>	620 (316–1404)	591 (357–946)	521 (320–914)
Tocopherol intake (mg/d)	13.1 (2.1–40.2)	12.3 (2.1–26.2)	13.8 (5.8–40.2)	NA	NA
Retinol intake (μg/d)	1622 (434–5806)	1767 (434–5806)	1523 (480–4674)	NA	NA
Ascorbate intake (mg/d)	112 (9–327)	124 (9–240)	104 (19–327)	NA	NA
Selenium intake (μg/d)	40 (6–113)	39 (6–105)	43 (8–113)	NA	NA

<sup>1</sup>Median (range). Hb, hemoglobin; NA, not available.

<sup>2,7</sup>Significantly different from control group: <sup>2</sup>*P* < 0.001, <sup>7</sup>*P* ≤ 0.005.

<sup>3,4,8,9</sup>Significantly different from HPA group: <sup>3</sup>*P* < 0.001, <sup>4</sup>*P* = 0.025, <sup>8</sup>*P* = 0.029, <sup>9</sup>*P* < 0.005.

<sup>5,6,10</sup>Significantly different from group 2: <sup>5</sup>*P* < 0.001, <sup>6</sup>*P* = 0.005, <sup>10</sup>*P* = 0.048.

might be related to the increased free radical damage in PKU. In the present study, we evaluated the possible relation between low serum Q<sub>10</sub> concentrations (already shown in a smaller group of patients with PKU) and other lipophilic antioxidants (tocopherol and retinol), selenium, GPX activity, and malondialdehyde as a marker of lipid peroxidation.

## SUBJECTS AND METHODS

### Subjects

This was a cross-sectional study of 58 patients with PKU (median age: 13 y; range: 2–36 y; 25 males and 33 females) under dietary treatment, 58 age-matched control subjects (median age: 12 y; range: 1–38 y; 27 males and 31 females), and 30 children with moderate hyperphenylalaninemia (HPA; plasma phenylalanine < 360 μmol/L) consuming an unrestricted diet (median age: 7.5 y; range: 3–17 y; 11 males and 19 females). The patients with PKU were following a phenylalanine-restricted diet supplemented with a tyrosine-enriched amino acid mixture (Analog XP in infancy, Maxamum XP in childhood, and Maxamum XP in adolescence and adulthood; Scientific Hospital Supplies, Barcelona, Spain).

To better understand the relation between Q<sub>10</sub> and the other variables, we stratified the PKU group according to serum Q<sub>10</sub> concentrations as follows. Group 1 contained 25 patients (aged 2–36 y; median: 16 y) with serum Q<sub>10</sub> concentrations below the 10th percentile of control values (< 0.45 μmol/L; median: 0.37 μmol/L; range: 0.20–0.45 μmol/L). Group 2 contained 33 patients (aged 2–35 y; median: 13 y) with serum Q<sub>10</sub> concentrations > 0.45 μmol/L (median: 0.57 μmol/L; range: 0.46–1.67 μmol/L).

Samples from the patients with PKU, the children with HPA, and the control group were obtained while the subjects were in a fasting state. Informed consent was obtained from the adults and adolescents with PKU and from the parents of the children with PKU. Samples from patients and control subjects were obtained in accordance with the Helsinki Declaration of 1964, as revised in

1996. The Ethics Committee of the Hospital Sant Joan de Déu approved the study.

### Methods

Plasma phenylalanine was analyzed by ion-exchange chromatography with an LKB Biochrom 20 analyzer (Pharmacia Biotech, Cambridge, United Kingdom). Serum Q<sub>10</sub> concentrations were analyzed by HPLC (Serie 200; Perkin Elmer, Norwalk, CT) with electrochemical detection (Coulchem II detector; ESA, Chelmsford, MA), according to a previously reported procedure (12).

Serum retinol, serum tocopherol, and plasma malondialdehyde (13, 14) were analyzed by HPLC (Serie 200; Perkin Elmer) with ultraviolet detection. Plasma ascorbate was analyzed in a Cobas Fara centrifugal analyzer (Roche Diagnostic Systems, Rotkreuz, Switzerland) by an automated enzymatic procedure (15). Serum selenium was assayed by hydride generation and atomic absorption spectrophotometry (16). Erythrocyte GPX activities were measured in a Cobas Fara analyzer according to the Plagia and Valentine method (17).

Daily intakes of tocopherol, retinol, ascorbate, and selenium were calculated for the patients with PKU with the use of the SANUTRIN V2.0 program (Novartis, Barcelona, Spain) from data obtained through a 3-d dietary questionnaire.

### Statistical methods

The Kolmogorov-Smirnov test was applied to check the distribution of the data. Because the data did not follow a Gaussian distribution, the Mann-Whitney test was applied for statistical comparisons and the Spearman test for correlations. The Bonferroni correction was applied for multiple comparisons. Statistical studies were performed with the SPSS program (version 10.0; SPSS Inc, Chicago).

## RESULTS

Serum Q<sub>10</sub> concentrations were significantly lower in the combined PKU group than in the HPA and control groups (Table 1).



Plasma malondialdehyde concentrations were significantly higher in the combined PKU group than in the control group. No significant differences were observed in retinol, tocopherol, or ascorbate concentrations between the combined PKU group and the other groups. However, 10 of the 58 (17.2%) patients with PKU had low tocopherol concentrations ( $< 15.1 \mu\text{mol/L}$ ). Plasma selenium values were significantly lower in the combined PKU group than in the HPA and control groups, whereas GPX activity did not differ significantly between groups (Table 1). No significant differences were observed between the HPA and control groups in any of the variables studied, except for plasma phenylalanine concentrations ( $P < 0.001$ ).

In the patients with PKU, significantly positive correlations were observed between  $Q_{10}$  and tocopherol ( $r = 0.510$ ,  $P < 0.001$ ), between GPX and plasma selenium concentrations ( $r = 0.337$ ,  $P = 0.007$ ), and between daily selenium intake and plasma selenium concentrations ( $r = 0.364$ ,  $P = 0.031$ ). No significant correlations were observed for the other variables of the study (plasma concentrations of vitamins, phenylalanine, and malondialdehyde and daily intakes of vitamins and selenium) in the PKU group.

When the patients with PKU were stratified into 2 groups according to  $Q_{10}$  values, tocopherol concentrations were significantly lower in group 1 than in group 2, the HPA group, and the control group, whereas malondialdehyde concentrations were significantly higher (Table 1). Thirty-two percent (8 of 25) of the patients in group 1 had low tocopherol concentrations, in contrast with 6% (2 of 33) of the patients in group 2 (chi-square test: 6.72,  $P < 0.001$ ). No significant differences were observed in daily intakes of tocopherol, retinol, ascorbate, and selenium; plasma GPX activity; or plasma concentrations of retinol, selenium, and phenylalanine between groups 1 and 2. Additionally, no significant differences were observed in concentrations of malondialdehyde, tocopherol, retinol, or ascorbate between group 2, the HPA group, and the control group.

## DISCUSSION

Antioxidant disturbances have been described in PKU (4, 5), and these alterations have been related to a dysfunction in GPX activity caused by selenium deficiency. Despite selenium supplementation in the present study, plasma selenium concentrations in the combined PKU group remained significantly lower than those in the HPA and control groups, but GPX activity did not differ significantly.


Although there are some studies of lipophilic antioxidants in PKU (3, 18, 19), to our knowledge, the relation between them and lipid peroxidation has not been reported. According to our results, malondialdehyde concentrations were higher in the combined PKU group than in the HPA and control groups, suggesting an increased lipid peroxidation in the patients. Decreased plasma concentrations of either selenium or  $Q_{10}$  might be involved in the increased lipid peroxidation. Although tocopherol was not significantly lower in the PKU group as a whole, 17.2% of patients had suboptimal concentrations of this vitamin, suggesting a relation between low tocopherol and high malondialdehyde values. Furthermore, correlation analysis showed a significantly positive association between plasma concentrations of tocopherol and  $Q_{10}$ . The relation between the 2 antioxidants was previously reported by other authors (20), suggesting a close interaction between  $Q_{10}$  and tocopherol. Noack et al (21) showed a relation between tocopherol depletion and  $Q_{10}$  during lipid peroxidation in mitochondria.

$Q_{10}$  concentrations are low in some patients with PKU (8). In a previous study (9), low  $Q_{10}$  values were mainly associated with high plasma phenylalanine concentrations in a well-controlled PKU population (plasma phenylalanine concentrations ranged between 205 and 643  $\mu\text{mol/L}$ ; median: 341  $\mu\text{mol/L}$ ). In the present study, we stratified the patients into 2 groups according to  $Q_{10}$  concentrations to assess the relation between  $Q_{10}$  concentrations and the other variables in PKU. Although daily tocopherol intake was not significantly different between the 2 groups of patients with PKU, serum tocopherol concentrations were significantly lower in group 1 than in the other groups, suggesting a higher tocopherol consumption in patients with deficient  $Q_{10}$  status than in those with normal  $Q_{10}$  values. Moreover, malondialdehyde concentrations were significantly higher in group 1 than in the other groups. This finding supports a relation between low  $Q_{10}$  values, increased tocopherol consumption, and a higher degree of lipid peroxidation in this group of patients with PKU.

Selenium deficiency could also be involved in this increased lipid peroxidation (4). However, daily selenium intake, plasma selenium concentrations, and GPX activity were not significantly different between the patients with PKU with  $Q_{10}$  deficiency and those with normal serum  $Q_{10}$  concentrations. It is probable that both selenium and  $Q_{10}$  are involved in oxidative stress in patients with PKU by different mechanisms. However, the normal GPX activity found in these patients and the fact that GPX and selenium were not significantly different between the 2 PKU groups suggests that plasma selenium deficiency is not a main factor in lipid peroxidation, at least among our patients.

The dietary supplementation with selenium and other vitamins was on the same order of magnitude as reported in other studies (3) and was always higher than the recommended dietary allowances. Furthermore, no significant differences were observed in dietary intakes of vitamins and selenium between the PKU groups. Other authors reported normal tocopherol values in selenium-deficient patients with PKU despite a high supplementation with this vitamin (4). To explain these findings, those authors suggested a higher rate of tocopherol oxidation in a selenium-deprived state. In our group of patients, we also observed low-normal values of tocopherol despite dietary supplementation with this vitamin and selenium. Probably, dietary supplementation of tocopherol was lower in our PKU group than that reported by van Bakel et al (4).

The correction of plasma tocopherol concentrations in patients with PKU with suboptimal concentrations of this vitamin seems advisable. However some studies have shown that  $\alpha$ -tocopherol can act either as an antioxidant or as a prooxidant for the lipoprotein lipids (20, 22), and the presence of  $Q_{10}$  can suppress the prooxidant or complement the antioxidant effect of tocopherol. Therefore, supplementation not only with tocopherol but also with  $Q_{10}$  may be a better way of preventing peroxidative damage in these patients. Further investigations may be needed to assess the effect of this supplementation both on  $Q_{10}$  concentrations and on lipid peroxidation status in patients with PKU.

In summary, plasma lipid peroxidation seems to be increased in PKU. Low values of  $Q_{10}$  could be associated with both excessive tocopherol consumption and high malondialdehyde concentrations in patients with PKU. The correction of either tocopherol or low  $Q_{10}$  values seems advisable to prevent increased lipid peroxidation in some patients with PKU. 



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