Decreased serum ubiquinone-10 concentrations in phenylketonuria[1,2]

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ABSTRACT

Background: Ubiquinone-10 is a lipid with important metabolic functions that may be decreased in phenylketonuria (PKU) because patients with PKU consume diets restricted in natural proteins.

Objective: We studied serum ubiquinone-10 concentrations in PKU patients.

Design: This was a retrospective, transversal study in which we compared serum ubiquinone-10, plasma cholesterol, plasma tyrosine, and plasma phenylalanine concentrations in 43 PKU patients with concentrations in a reference population (n = 102). Serum ubiquinone-10 concentrations were analyzed by HPLC with ultraviolet detection. Plasma tyrosine and phenylalanine were measured by ion-exchange chromatography.

Results: Serum ubiquinone-10 concentrations in PKU patients were significantly lower than in the reference population ($P < 0.01$ for patients aged 1 mo to <8 y and $P < 0.00005$ for patients aged 8–33 y). Moreover, 5 of 18 PKU patients (28%) in the younger age group and 10 of 23 (43%) in the older age group had serum ubiquinone-10 concentrations below the reference interval.

Conclusions: Serum ubiquinone-10 deficiency appears to be related to the restricted diet of PKU patients. Because serum ubiquinone-10 plays a major antioxidant role in the protection of circulating lipoproteins, the correction of ubiquinone-10 concentrations should be considered in PKU patients. Further investigation seems advisable to elucidate whether the deficiency in serum ubiquinone-10 status is clinically significant. *Am J Clin Nutr* 1999;70:892–5.

KEY WORDS Phenylketonuria, ubiquinone-10, tyrosine, phenylalanine, cholesterol, dietetic treatment, humans

INTRODUCTION

Phenylketonuria [PKU; McKusick number 261600 (1)] 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 (2). Treatment of PKU patients consists of restriction of natural proteins and supplementation with a phenylalanine-free, tyrosine-enriched amino acid mixture (3). Despite treatment, however, low plasma tyrosine concentrations have been reported in PKU patients, especially after an overnight fast (3). Other important nutrients, such as vitamins, minerals (4), and carnitine (5), may also be diminished because of this restricted diet.

Ubiquinone-10 is a lipid that has been implicated in several biological functions. In tissues, it has an important role in energy transduction in mitochondria. Moreover, in its reduced form (ubiquinol), it protects cells from peroxidative damage (6). In blood, ubiquinone-10 is transported by lipoproteins and it is probably the most important antioxidant within LDL (7). Ubiquinone-10 is synthesized through 2 metabolic pathways: the quinone moiety of ubiquinone is synthesized predominantly from tyrosine, whereas the polyprenyl side chain is synthesized from acetyl CoA through the mevalonate pathway common for cholesterol synthesis (6). Tyrosine availability seems, therefore, to be an important factor for ubiquinone-10 biosynthesis, especially in PKU patients. Moreover, dietary sources may contribute up to 25% of total serum ubiquinone-10 (8). Foods such as poultry and meat, which are restricted for PKU patients, may be critical for maintaining optimal serum ubiquinone-10 concentrations (8).

Our aim was to investigate a possible deficiency of serum ubiquinone-10 in a group of PKU patients periodically evaluated in our hospital (a reference center for PKU in Catalonia). We also selected some related metabolites (plasma cholesterol, tyrosine, and phenylalanine) to search for the origin of this hypothetical deficiency.

SUBJECTS AND METHODS

We studied 43 PKU patients who had been periodically evaluated in our hospital. The patients ranged in age from 1 mo to 33 y (median: 13 y); 24 were female and 19 were male. We compared our results for the patient population with reference values for a healthy population (n = 102; 38 females and 64 males ranging in age from 1 mo to 35 y) (9). The clinical history and analytic values of subjects in the reference population were reviewed to ensure that these subjects were healthy; additionally, the reference population was matched with the patients by age and sex. Because

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serum ubiquinone-10 concentrations in our reference population were dependent on age but not sex (9), reference values and patient results were grouped according to age for statistical comparison. The 2 age groups were from 1 mo to <8 y (reference population, $n = 62$; PKU patients, $n = 18$) and from 8 to 33 y (reference population, $n = 40$; PKU patients, $n = 25$). Because our adult patients were all women, we established a unique reference interval for plasma tyrosine (tyrosine concentrations in healthy women and children are similar). All specimens were collected after subjects had fasted overnight (9–10 h). Samples from patients and from the reference population were analyzed simultaneously in the same laboratory. All PKU patients were supplemented with a tyrosine-enriched amino acid mixture (Analog XP in infancy, Maxamaid XP in childhood, and Maxamun XP in adolescence and adulthood; Scientific Hospital Supplies, Barcelona, Spain). This study was performed in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Total ubiquinone-10 (reduced plus oxidized) concentrations were measured by HPLC (Integral 4000; Perkin-Elmer, Norwalk, CT) with ultraviolet detection (275 nm) according to the method of Zierz et al (10) with some modifications (9). Serum ubiquinone-10 results are reported as molar concentrations (μmol/L) and in relation to cholesterol (μmol/mol cholesterol) (9). Plasma amino acids (tyrosine and phenylalanine) were measured by ion-exchange chromatography (LKB analyzer; Pharmacia, Uppsala, Sweden). Cholesterol was measured according to standard procedures with the Cobas Integra Analyzer (Roche Diagnostic Systems, Basel, Switzerland).

The Kolmogorov-Smirnov test was applied to check the distribution of the data in both populations. Because the data did not follow a Gaussian distribution, the Mann-Whitney *U* test was applied for statistical comparisons and the Spearman test for correlations. Reference intervals were established as medians and percentiles 2.5 and 97.5. Statistical analyses were performed with STATGRAPHICS (version 6.0; Manugistics Inc, Rockville, MD).

RESULTS

Serum ubiquinone-10 concentrations were significantly lower in PKU patients than in the reference population (**Table 1**). Moreover, 5 of 18 PKU patients (28%) in the younger age group and 10 of 23 (43%) in the older age group had serum ubiquinone-10 concentrations below the reference interval. When concentrations of ubiquinone were related to those of cholesterol, significant differences between patient and reference values were also observed (Table 1). Plasma cholesterol, tyrosine, and phenylalanine concentrations in the PKU patients and the reference population, and statistical comparisons of the results, are also summarized in Table 1.

The Spearman test showed positive correlations between serum ubiquinone-10 and cholesterol concentrations in both the reference population and the PKU patients (**Figure 1**). Additionally, negative correlations were observed between ubiquinone-10 concentrations and age in PKU patients ($r = -0.36$, $P < 0.05$) and in the reference population ($r = -0.383$, $P < 0.001$). No correlations were observed between plasma tyrosine and serum ubiquinone-10 concentrations, tyrosine daily intake and ubiquinone-10 concentrations, or plasma phenylalanine and ubiquinone-10 concentrations in either group.

DISCUSSION

Ubiquinone-10 has important functions in mitochondrial energy metabolism. The first of these is gathering electrons from the substrates NADH and succinate for complex III of the respiratory chain (11); the second is establishing a proton gradient across the mitochondrial membrane that can be coupled to ATP production (12). Moreover, an important role for ubiquinone-10 in the antioxidant system has been recognized (7). Ubiquinone deficiency has been identified in several conditions, such as cancer (13), mitochondrial disease (14, 15), and mevalonate kinase deficiency (16), but not in PKU. In our results, however, low serum ubiquinone-10 concentrations were common in PKU patients. This deficiency may be caused by several mechanisms. First, PKU patients may have insufficient dietary intakes of ubiquinone-10 [intakes of meat and poultry, foods with high concentrations of ubiquinone (8), are restricted for patients with PKU] and cholesterol, concentrations of which were also significantly lower in the PKU patients than in the reference population, as observed by others (17). Moreover, cholesterol concentrations correlated well with serum ubiquinone-10 concentrations in both PKU patients and the reference population. Second, tyrosine availability in tissues may be diminished in patients with PKU, causing a deficiency in the endogenous synthe-

TABLE 1
Serum ubiquinone-10, cholesterol, tyrosine, and phenylalanine concentrations in patients with phenylketonuria (PKU) and in a reference population[1]

	PKU patients ($n = 43$)	Reference population ($n = 102$)
Ubiquinone-10 (μmol/L)		
1 mo to <8 y	0.60 (0.29–1.0)[2]	0.80 (0.46, 1.38)
8–18 y	0.37 (0.16–0.87)[3]	0.57 (0.34, 1.03)
Ubiquinone-10 (μmol/mol cholesterol)		
1 mo to <8 y	171 (97–276)[4]	203 (137, 341)
8–18 y	117 (51–264)[5]	169 (111, 248)
Cholesterol (mmol/L)		
<14 y	3.3 (2.4–5.0)[2]	3.9 (2.5, 5.2)
14–33 y	3.1 (2.3–3.9)[3]	4.3 (2.8, 6.2)
Tyrosine (μmol/L)		
1 mo to 35 y	52 (31–92)	66 (46, 87)
Phenylalanine (μmol/L)		
1 mo to 35 y	375 (59–1044)[6]	50 (43, 58)

[1] Median with range in parentheses for PKU patients and percentiles 2.5 and 97.5 in parentheses for the reference population.
[2–6] Significantly different from the reference population (Mann-Whitney *U* test): [2] $P < 0.01$, [3] $P < 0.00005$, [4] $P < 0.05$, [5] $P < 0.001$, [6] $P < 0.00000005$.

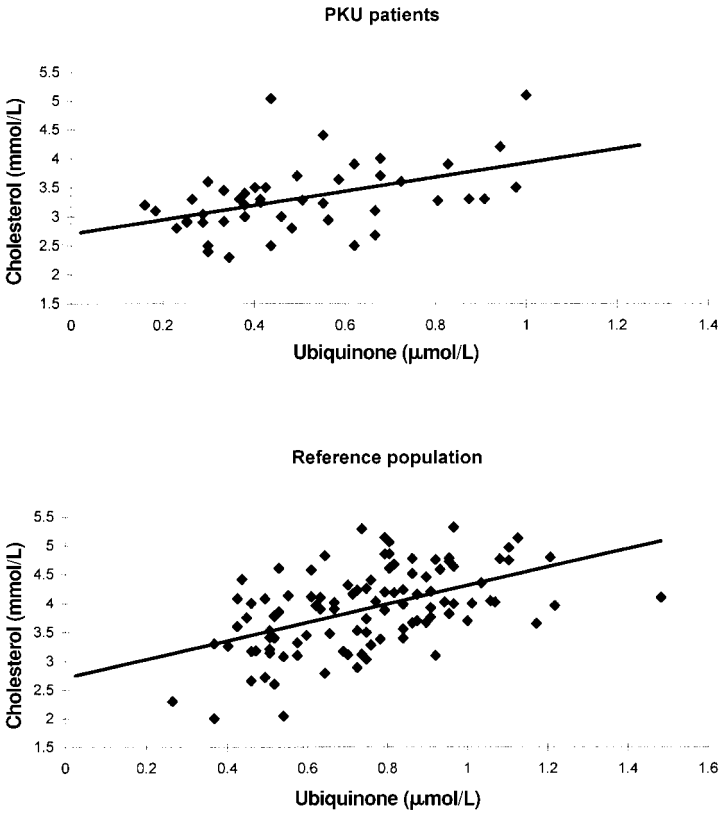

FIGURE 1. Correlation between serum ubiquinone-10 and plasma cholesterol concentrations in patients with phenylketonuria (PKU) (r = 0.503, P < 0.001) and in a reference population (r = 0.485, P < 0.00001).

sis of ubiquinone-10. Although plasma tyrosine concentrations in our PKU patients were within the normal range after an overnight fast, we observed significant differences between the patients and the reference population when serum ubiquinone-10 concentrations were expressed in relation to cholesterol. This suggests that the low serum ubiquinone-10 concentrations were due not only to low dietary intakes of ubiquinone-10 in PKU patients, but also to a possible tyrosine deficiency in tissues, with a resulting reduced endogenous synthesis of ubiquinone-10.

Ubiquinone-10 deficiency was found in only some of the well-controlled PKU patients and was more prevalent in the older age group (the older patients had been under strict dietary control for several years). In contrast, adolescent patients with poor dietary control (6 PKU patients not included in this study) did not have low serum ubiquinone-10 concentrations (0.48–1.25 μmol/L), lending support to the idea that ubiquinone deficiency in PKU patients is mainly due to their special diet.

A third mechanism probably involved in ubiquinone deficiency in PKU is the inhibition of the rate-limiting enzymes of cholesterol synthesis (especially hydroxymethylglutaryl-CoA reductase), as shown in experimental hyperphenylalaninemia (18). However, no correlations were observed between phenylalanine and ubiquinone-10 concentrations in serum in PKU patients in the present study, suggesting that this mechanism was not a determining factor in serum ubiquinone-10 deficiency in these patients. This hypothesis needs to be tested further. In all likelihood, ubiquinone deficiency is the result of the sum of the 3 mechanisms described above.

The clinical significance of our findings is not entirely clear. However, because serum ubiquinone-10 plays a major antioxidant role in the protection of circulating lipoproteins, the correction of its concentrations needs to be considered. Further research seems advisable to elucidate whether this deficient status is also present in tissues and, if so, whether tissue deficiency results in altered ubiquinone-10 metabolism. If serum ubiquinone-10 deficiency is an indication of a deficiency of ubiquinone-10 in cells, several adverse consequences may result. First, a subtle ubiquinone-10 deficiency maintained throughout life may impair electron transport through the mitochondrial respiratory chain, increasing free radical generation. Additionally, the reduced amounts of ubiquinone-10 in tissues and serum would be unable to cope with excess free radical production. However, antioxidant metabolism in blood cells and plasma is normal in PKU patients according to our observations (19, 20). This may be attributable to the use of some important supplementary antioxidant components in our PKU patients (such as tocopherol and selenium).

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REFERENCES

1. McKusick V. Mendelian inheritance in man. 8th ed. Baltimore: Johns Hopkins University Press, 1988.
2. Scriver CR, Kaufman S, Woo SLC. The hyperphenylalaninemias. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic

and molecular basis of inherited disease. 7th ed. New York: McGraw Hill, 1995:147–60.
3. van Spronsen FJ, van Dijk T, Smit GPA, et al. Large daily fluctuations in plasma tyrosine in treated patients with phenylketonuria. Am J Clin Nutr 1996;64:916–21.
4. Gropper SS, Naglak MC, Nardella M, Plyler A, Rarback S, Yannicelli S. Nutrient intakes of adolescents with phenylketonuria and infants and children with maple syrup urine disease on semisynthetic diets. J Am Coll Nutr 1993;12:108–14.
5. Vilaseca MA, Briones P, Ferrer I, et al. Controlled diet in phenylketonuria may cause serum carnitine deficiency. J Inherit Metab Dis 1993;16:101–4.
6. Ernster L, Dallner G. Biochemical, physiological and medical aspects of ubiquinone function. Biochim Biophys Acta 1995;1271: 195–204.
7. Stocker R, Bowry VW, Frei B. Ubiquinol-10 protects human low-density lipoprotein more efficiently against lipid peroxidation than does α-tocopherol. Proc Natl Acad Sci U S A 1991;88:1646–50.
8. Weber C, Bysted A, Holmer G. The coenzyme Q10 content of the average Danish diet. Int J Vitam Nutr Res 1997;67:123–9.
9. Artuch R, Moreno J, Quintana M, Puig RM, Vilaseca MA. Serum ubiquinone-10 in a pediatric population. Clin Chem 1998;44:2378–9.
10. Zierz S, Von Wersebe O, Bleistein J, Jerusalem F. Exogenous coenzyme Q (CoQ) fails to increase CoQ in skeletal muscle of two patients with mitochondrial myopathies. J Neurol Sci 1990;95:283–90.
11. Crane FL, Sun IL, Sun EE. The essential functions of coenzyme Q. Clin Invest 1993;71:S5-59.
12. Mitchell P. The vital protonmotive role of coenzyme Q. In: Folkers K, Littarru GP, Yamagami T, eds. Biomedical and clinical aspects of coenzyme Q. Vol 6. Amsterdam: Elsevier, 1991:3–10.
13. Folkers K, Osterborg A, Nylander M, Morita M, Mellstedt H. Activities of vitamin Q10 in animal models and a serious deficiency in patients with cancer. Biochem Biophys Res Commun 1997;234:296–9.
14. Zierz S, Jahns G, Jerusalem F. Coenzyme Q in serum and muscle of 5 patients with Kearns-Sayre syndrome and 12 patients with ophthalmoplegia plus. J Neurol 1989;236:97–101.
15. Sobreira C, Hirano M, Shanske S, et al. Mitochondrial encephalomyopathy with coenzyme Q10 deficiency. Neurology 1997;48: 1238–43.
16. Hübner C, Hoffmann GF, Charpentier C, et al. Decreased plasma ubiquinone-10 concentration in patients with mevalonate kinase deficiency. Pediatr Res 1993;34:129–33.
17. Schulpi KH, Scarpalezou A. Triglycerides, cholesterol, HDL, LDL and VLDL cholesterol in serum of phenylketonuric children under dietary control. Clin Pediatr 1989;28:466–9.
18. Castillo M, Zafra MF, García-Peregrín E. Inhibition of brain and liver 3-hydroxy-3-methylglutaryl-CoA reductase and mevalonate-5-pyrophosphate decarboxylase in experimental hyperphenylalaninemia. Neurochem Res 1988;13:551–5.
19. Moyano D, Vilaseca MA, Pineda M, et al. Tocopherol in inborn errors of intermediary metabolism. Clin Chim Acta 1997;263:147–55.
20. Sierra C, Vilaseca MA, Moyano D, Brandi N, Campistol J, Lambruschini N. Antioxidant status in hyperphenylalaninemia. Clin Chim Acta 1998;276:1–9.

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