Antioxidant and thyroid hormone status in selenium-deficient phenylketonuric and hyperphenylalaninemic patients

Marit ME van Bakel, Gert Printzen, Bendicht Wermuth, and Ulrich N Wiesmann

ABSTRACT

Background: Subjects consuming protein-restricted diets, such as patients with phenylketonuria (PKU) or milder hyperphenylalaninemia (HPAs) are at risk of selenium deficiency. Selenium is a cofactor of the antioxidant enzyme glutathione peroxidase and of the thyroid hormone converting enzyme thyroxine deiodinase.

Objective: Our goal was to investigate the effects of low plasma selenium on antioxidant and thyroid hormone status.

Design: We assessed plasma selenium, plasma total antioxidant status and the individual components thereof, erythrocyte antioxidant status, and plasma thyroid hormones in 24 PKU and 10 HPA patients and in 42 age-matched control subjects.

Results: Selenium was significantly lower in both PKU and HPA patients than in control subjects and the PKU patients had lower values than did the HPA patients. Total antioxidant status was lower in both patient groups than in the control group, whereas α-tocopherol, albumin, and uric acid were not significantly different among groups. Plasma selenium correlated well (r = 0.76) with erythrocyte glutathione peroxidase. PKU patients had lower glutathione peroxidase activity than did HPA patients and control subjects and lower glutathione concentrations than did control subjects. Both patient groups had lower superoxide dismutase activity than did control subjects. Free triiodothyronine was higher in both patient groups than in control subjects, whereas free thyroxine was higher in the PKU patients only. Free thyroxine and reverse triiodothyronine were inversely correlated with selenium.

Conclusion: Supplementation with selenium seems to be advisable for patients consuming diets low in natural protein.


KEY WORDS Phenylketonuria, hyperphenylalaninemia, selenium deficiency, low-protein diet, antioxidant status, thyroid hormone status, glutathione peroxidase, glutathione

INTRODUCTION

Hyperphenylalaninemia (HPA) is the most common inherited disorder of amino acid metabolism and arises from the single enzyme deficiency of phenylalanine hydroxylase (phenylalanine 4-monoxygenase), which converts the essential amino acid phenylalanine to tyrosine. Failure of this conversion results in the buildup of phenylalanine in blood, ranging from mild HPA to classic phenylketonuria (PKU). Elevated concentrations of phenylalanine and its metabolites interfere with normal development of the central nervous system, leading to severe mental retardation. Therapy consists of a diet made low in phenylalanine by eliminating high-protein foods, enabling children with PKU to develop normally (1, 2). As a substitute for proteins, PKU patients consume an artificial amino acid mixture that does not contain phenylalanine (eg, Milupa PKU 1-3; Milupa AG, Friedrichsdorf, Germany); such mixtures are enriched with vitamins and minerals but do not contain extra selenium.

Normally, selenium is consumed as part of protein; thus, children consuming low-protein diets are at risk of developing selenium deficiency (3–5). Selenium functions as a cofactor of 2 functionally distinct enzymes: glutathione peroxidase (EC 1.11.1.9) (6, 7) and 5′-deiodinase type I (thyroxine deiodinase; EC 3.8.1.4) (8). Selenium-dependent glutathione peroxidase and other antioxidants are involved in the elimination of free radicals and reactive oxygen species (ROS). Highly reduced nutritional intake of selenium may impair enzyme activity and thus have deleterious effects on not only cells and organs but the whole organism (9, 10). Thyroxine deiodinase is responsible for the conversion of the prohormone thyroxine (T4 or 3′,3′,5′,3′-tetraiodothyronine) into the biologically active triiodothyronine (T3 or 3′,3′,5′-triiodothyronine) and of the inactive reverse triiodothyronine (rT3 or 3′,3′,5′-triiodothyronine) into diiodothyronine (T3 or 3′,3′-diiodothyronine). Thyroid hormones are important for normal growth and development of the maturing organism (11).

The aim of this study was to determine whether reduced plasma selenium in PKU and HPA patients results in 1) changes in total plasma antioxidant status or in individual components thereof (eg, uric acid, albumin, and α-tocopherol); 2) changes in cellular activity of glutathione peroxidase, reduced glutathione (GSH), and other enzymes involved in the ROS defense of erythrocytes; or 3) changes in plasma thyroid hormones resulting from reduced thyroxine deiodinase activity, and 4) whether these changes are correlated with the extent of the selenium reduction.
SUBJECTS AND METHODS

Subjects

The study included 24 PKU patients (14 boys and 10 girls; phenylalanine tolerance < 12 mg phenylalanine \( \text{kg}^{-1} \cdot \text{d}^{-1} \) aged 9.65 ± 4.06 y (range: 4.13–17.88 y), 10 HPA patients (5 boys and 5 girls; phenylalanine tolerance > 12 mg phenylalanine \( \text{kg}^{-1} \cdot \text{d}^{-1} \) aged 9.08 ± 5.17 y (range: 4.09–18.24 y), and 42 control subjects (30 boys and 12 girls) aged 11.18 ± 4.84 y (range: 1.62–19.06 y). Blood samples were collected between 4 and 6 times per subject over a period of 14 mo. The CVs of the variables measured over this time period were < 10%, except for the CVs for activities of superoxide dismutase (SOD) and glutathione peroxidase, which were occasionally up to 19% (for 2 and 3 patients, respectively). Written consent was given by the parents of the subjects and the study was approved by the ethics committee of Bern.

Collection of blood samples

Blood samples were collected in tubes containing EDTA or lithium-heparin (for measurement of selenium only) as an anticoagulant (Sarstedt AG, Sevelen, Switzerland). After centrifugation (10 min, 1000 × g, 4°C), plasma was separated and frozen at −20°C until used. The cells were centrifuged (7 min, 350 × g, 4°C) and washed 3 times with 0.9% NaCl. The erythrocytes were processed immediately for measurement of GSH. For measurement of enzyme activity, 20 μL of the washed erythrocytes was lysed with 1180 μL of a cold Triton X-100 solution (0.5% by wt; Merck AG, Zurich, Switzerland). After 15 min on ice, the tubes were centrifuged (5 min, 6000 × g, 4°C) and the supernate was then transferred to Cobas tubes.

Plasma selenium

Plasma selenium concentrations were measured according to the method of Rückgauer et al (12) by electrothermal atomic absorption spectrophotometry (Zeemann 4100 ZL with graphite furnace HGA 600 and an AS 70 autosampler; Perkin-Elmer, Überlingen, Germany) with a palladium-iridium-magnesium matrix modifier. The values were calculated by using a standard curve with a palladium-iridium-magnesium furnace HGA 600 and an AS 70 autosampler; Perkin-Elmer, Überlingen, Germany) with a palladium-iridium-magnesium matrix modifier. The values were calculated by using a standard curve, the selenium atomic absorption spectrophotometry standard (Alfa Aesar, Johnson & Matthey GmbH, Karlsruhe, Germany) with a palladium-iridium-magnesium matrix modifier. The values were calculated by using a standard curve, the selenium atomic absorption spectrophotometry standard (Alfa Aesar, Johnson & Matthey GmbH, Karlsruhe, Germany) with a palladium-iridium-magnesium matrix modifier.

Reduced glutathione in erythrocytes

GSH was measured fluorometrically (Perkin-Elmer model 3000) according to the method of Hissin and Hilf (17), with some modifications. Briefly, 30 μL erythrocytes were lysed with 0.5 mL double-distilled water. Proteins were precipitated by the addition of 0.5 mL of a metaphosphoric acid solution [1.67 g metaphosphoric acid, 0.20 g EDTA (disodium salt), and 30.0 g NaCl in 100 mL double-distilled water; Merck AG]. After 5 min on ice, the protein precipitate was separated from the remaining solution by centrifugation in an Eppendorf centrifuge (10 min, 3000 × g, 4°C). Of the supernate, 100 μL was added to 1.8 mL of a 0.1-mol/L sodium phosphate buffer, pH 8.0, containing 5 mmol EDTA/L. After the addition of 100 μL of 1% α-phthalaldehyde (Merck AG) in methanol, the reaction mixture was incubated at room temperature for exactly 15 min. Fluorescence was determined at 420 nm with activation at 341 nm. The concentrations of the samples were calculated by using a standard curve, with GSH ranging from 3.91 to 62.5 μmol/L (Sigma AG, Buchs, Switzerland), and were corrected for the scale expansion factor of the highest standard and related to the amount of hemoglobin.

Antioxidants in plasma

α-Tocopherol in plasma was measured by an HPLC method with ultraviolet absorbance detection according to Kock et al (18). The concentrations of the unknown samples were calculated by using a standard curve with all-rac-α-tocopherol (Sigma AG) values of 5.09, 15.27, 30.53, and 61.07 mg/L and were corrected for recovery of an internal standard of 15 mg α-tocopherol acetate/L (Merck AG). The interassay CV with use of a serum pool was 5.8%; the intraassay CV was 1.8%.

Albumin and uric acid in plasma were measured spectrophotometrically (Hitachi 917; Roche Diagnostics AG) according to the manufacturer’s instructions (Wako, Zurich, Switzerland and Roche, respectively). The inter- and intraassay CVs of both methods were < 2%.

Plasma total antioxidant status was measured spectrophotometrically with the Randox total antioxidant status kit (Randox Laboratories Ltd, Crumlin, United Kingdom) on a Cobas Mira Plus (Roche) according to the manufacturer’s instruction. The interassay CV was 1.2%.

Thyroid hormones in plasma

Free thyroxine (FT₄) and free triiodothyronine (FT₃) concentrations were measured by competitive chemiluminescence immunoassays (Chiron Diagnostics Corporation, East Walpole, MA). With each run, Chiron Diagnostics ligand quality-control material, levels 1, 2, 3, were included.

rT₃ was measured by radioimmunoassay with a kit of Biochem Immunosystems GmbH (Freiburg, Germany). The interassay CV of the control serum included in the kit was < 7%. All thyroid hormone concentrations were measured on one day.

Statistics

Data were first analyzed for normality. Age, selenium, glutathione reductase, albumin, FT₄, rT₃, and total antioxidant status were normally distributed.
Plasma antioxidant variables and control subjects

<table>
<thead>
<tr>
<th></th>
<th>PKU patients (n = 24)</th>
<th>HPA patients (n = 10)</th>
<th>Control subjects (n = 42)</th>
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<tbody>
<tr>
<td>Selenium (µmol/L)</td>
<td>0.52 ± 0.20&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>0.92 ± 0.21&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1.23 ± 0.14</td>
</tr>
<tr>
<td>α-Tocopherol (µmol/L)</td>
<td>21.46 ± 4.06</td>
<td>19.25 ± 2.11</td>
<td>20.93 ± 6.15</td>
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<tr>
<td>Uric acid (µmol/L)</td>
<td>217.9 ± 50.4</td>
<td>235.7 ± 27.8</td>
<td>244.4 ± 104.8</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>39.8 ± 2.2</td>
<td>40.5 ± 2.3</td>
<td>39.5 ± 3.2</td>
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<tr>
<td>TAS (mmol/L)</td>
<td>1.52 ± 0.14&lt;sup&gt;4&lt;/sup&gt;</td>
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<sup>1</sup> x ± SD. PKU, phenylketonuria; HPA, hyperphenylalaninemia; TAS, total antioxidant status.

<sup>2</sup> Significantly different from control group [Dunn’s procedure for all multiple (pairwise) comparisons]; <sup>3</sup> P < 0.001; <sup>4</sup> P < 0.01.

<sup>3</sup> Significantly different from HPA group, P < 0.01 [Dunn’s procedure for all multiple (pairwise) comparisons].

<sup>4</sup> Significantly different from control group, P < 0.01 [Tukey’s test for multiple (pairwise) comparisons].

RESULTS

Plasma selenium and plasma antioxidants

Plasma values of selenium, α-tocopherol, uric acid, albumin, and total antioxidant status are listed in Table 1: correlations with plasma selenium and total antioxidant status in patients are shown in Table 2. Plasma selenium values of the PKU patients (range: 0.21–0.86 µmol/L) were significantly lower than those of the HPA patients (range: 0.64–1.21 µmol/L); additionally, both patient groups had significantly lower values than did the control subjects (range: 1.00–1.50 µmol/L). Plasma total antioxidant status was significantly lower in both PKU and HPA patients than in control subjects, but the patient groups did not differ significantly from each other. Of the plasma variables measured in the patients, only uric acid was significantly correlated with plasma selenium.

Erythrocyte antioxidant systems

Erythrocyte concentrations of GSH and the enzyme activities of glutathione peroxidase, SOD, glutathione reductase, and glutathione transferase are listed in Table 3: correlations with selenium and glutathione peroxidase in patients are shown in Table 2. The PKU patients had significantly lower glutathione peroxidase activity than did both the HPA patients and the control subjects. Only the PKU group had significantly lower GSH values than the control group, but both the PKU and HPA groups had significantly lower SOD activity than the control group.

The correlation between selenium in plasma and glutathione peroxidase in erythrocytes was highly significant (Figure 1). The curve was linear for selenium values less than ≈0.8 µmol/L and glutathione peroxidase values less than ≈45 U/g hemoglobin. Furthermore, GSH concentrations of the patients correlated with selenium. Although glutathione peroxidase correlated well with selenium, the other variables measured in erythrocytes were not correlated with glutathione peroxidase in patients.

Plasma thyroid hormones

The plasma concentrations of the thyroid hormones FT<sub>3</sub>, FT<sub>4</sub>, and rT<sub>3</sub>, and the ratios of FT<sub>4</sub> to FT<sub>3</sub> and of rT<sub>3</sub> to FT<sub>3</sub> are presented in Table 4: correlations with selenium in patients are shown in Table 2. PKU and HPA patients both had significantly higher FT<sub>3</sub> values than did control subjects, whereas FT<sub>4</sub> was significantly

Table 1

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<sup>4</sup> Significantly different from control group, P < 0.01 [Tukey’s test for multiple (pairwise) comparisons].

Table 2

Pearson’s product-moment correlations and P values of plasma and erythrocyte antioxidant variables and of thyroid hormone values with plasma selenium, of erythrocyte antioxidant variables with erythrocyte glutathione peroxidase activity, and of plasma antioxidant variables with plasma total antioxidant status in patients with phenylketonuria and hyperphenylalaninemia

<table>
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<tr>
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<th>Selenium</th>
<th>GSH peroxidase</th>
<th>TAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol (µmol/L)</td>
<td>−0.05</td>
<td>0.78</td>
<td>0.26</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>0.33</td>
<td>0.07</td>
<td>−0.28</td>
</tr>
<tr>
<td>Uric acid (µmol/L)</td>
<td>0.37</td>
<td>&lt;0.05</td>
<td>0.28</td>
</tr>
<tr>
<td>TAS (mmol/L)</td>
<td>0.18</td>
<td>0.33</td>
<td>0.01</td>
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<tr>
<td>FT&lt;sub&gt;3&lt;/sub&gt; (pmol/L)</td>
<td>−0.46</td>
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<td>0.14</td>
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<tr>
<td>FT&lt;sub&gt;4&lt;/sub&gt; (pmol/L)</td>
<td>0.27</td>
<td>0.14</td>
<td>0.01</td>
</tr>
<tr>
<td>FT&lt;sub&gt;4&lt;/sub&gt; FT&lt;sub&gt;3&lt;/sub&gt;</td>
<td>−0.55</td>
<td>0.001</td>
<td>0.11</td>
</tr>
<tr>
<td>FT&lt;sub&gt;3&lt;/sub&gt; FT&lt;sub&gt;4&lt;/sub&gt;</td>
<td>−0.56</td>
<td>&lt;0.0001</td>
<td>0.11</td>
</tr>
<tr>
<td>Erythrocyte</td>
<td>Glutathione peroxidase (U/g Hb)</td>
<td>0.76</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>Glutathione (µmol/g Hb)</td>
<td>0.40</td>
<td>&lt;0.05</td>
<td>0.32</td>
</tr>
<tr>
<td>Glutathione transferase (U/g Hb)</td>
<td>0.05</td>
<td>0.79</td>
<td>0.02</td>
</tr>
<tr>
<td>Glutathione reductase (U/g Hb)</td>
<td>−0.06</td>
<td>0.74</td>
<td>0.12</td>
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</table>

<sup>1</sup> n = 34. GSH, reduced glutathione; TAS, total antioxidant status; FT<sub>3</sub>, free triiodothyronine; FT<sub>4</sub>, free thyroxine; rT<sub>3</sub>, reverse triiodothyronine; Hb, hemoglobin; SOD, superoxide dismutase.
higher in PKU patients only. There were no significant differences between groups in the ratio of FT₄ to FT₃. In patients, FT₄ and rT₃ were higher in PKU patients only. There were no significant differences between groups in the ratio of FT₄ to FT₃. In patients, FT₄ and rT₃ were inversely correlated with plasma selenium concentrations.

DISCUSSION

Subjects

The patients’ diets were severely restricted in natural protein, but because they consumed an artificial amino acid mixture they had no nutritional deficiencies other than selenium deficiency. Thus, these patients constituted an ideal group for studying the effects of an isolated selenium deficiency of variable degree.

The severity of the plasma selenium deficiency correlated with the severity of the condition (PKU > HPA). Selenium deficiency results from multiple factors such as residual intake of natural protein, age, and duration of the restricted diet, although none of these variables alone correlated with plasma selenium.

Antioxidant defense

The total antioxidant capacity of a given compartment is divided into preventive antioxidants (eg, glutathione transferase) and chain-breaking antioxidants. Preventive antioxidants comprise scavenger enzymes, which convert potentially harmful ROS into less harmful products, and metal binding proteins (eg, ferritin, transferrin, ceruloplasmin, and albumin), which protect by sequestering transition metals. Chain-breaking antioxidants intercept damaging species and include enzymes such as SOD and glutathione peroxidase and nonenzymatic antioxidants such as α-tocopherol, GSH, uric acid, and albumin (19–21). Normally, the balance between the formation and the destruction of ROS is in equilibrium. However, impairment of the antioxidant system may result in oxidative stress. We assumed that our patients were not exposed to or did not generate more free radicals than do healthy children, but that their selenium deficiency reduced the capacity to cope with the usual free radical load.

Because plasma total antioxidant status, which represented a global value for the patients’ antioxidant status, was lower in both PKU and HPA patients than in control subjects, we measured α-tocopherol, albumin, and uric acid individually. Selenium, α-tocopherol, and uric acid were all positively correlated with total antioxidant status. Although these correlations were not significant (P = 0.33, 0.15, and 0.13, respectively), altogether, they may still account for the lower total antioxidant status of the patients.

α-Tocopherol is probably the most important lipid-soluble antioxidant protecting membranes, lipids, and lipoproteins. Our patients received an amino acid formula that was supplemented with vitamin E. The more restricted the diet, the higher the supply of vitamin E. In addition, the diet contained vitamin E–rich plant oils as nonprotein energy, resulting in an even higher vitamin E supply. The fact that plasma α-tocopherol concentrations in patients were not higher than those in control subjects may suggest a higher rate of oxidation of α-tocopherol in a selenium-deprived state.

The ROS scavenging activity of albumin is by nonspecific binding of transition metals and by the oxidation of albumin’s sulfhydryl groups. This may lead to an increased turnover of albumin. Even though significant differences in albumin concentrations were not found between groups, the tendency toward a positive correlation of albumin with selenium supports this idea.

| TABLE 3 |
| Antioxidant enzyme activities of the glutathione cycle and reduced glutathione in erythrocytes of patients and control subjects | PKU patients (n = 24) | HPA patients (n = 10) | Control subjects (n = 42) |
| GSH (μmol/g Hb) | 4.71 ± 0.64² | 6.17 ± 1.30 | 7.35 ± 2.32 |
| Glutathione peroxidase (U/g Hb) | 27.75 ± 12.30² | 49.59 ± 11.99 | 48.78 ± 7.46 |
| SOD (U/mg Hb) | 1.49 ± 0.55² | 1.57 ± 0.36 | 2.40 ± 0.33 |
| Glutathione reductase (U/g Hb) | 9.17 ± 1.72 | 9.67 ± 2.59 | 9.01 ± 2.01 |
| Glutathione transferase (U/g Hb) | 4.22 ± 1.69 | 4.11 ± 0.86 | 4.66 ± 1.50 |

¹ ± SD. PKU, phenylketonuria; HPA, hyperphenylalaninemia; Hb, hemoglobin; GSH, reduced glutathione; SOD, superoxide dismutase.
² Significantly different from control group [Dunn’s procedure for all multiple (pairwise) comparisons]: ³ P < 0.05, ⁴ P < 0.001.
³ Significantly different from HPA group, P < 0.05 [Dunn’s procedure for all multiple (pairwise) comparisons].

FIGURE 1. Correlation between erythrocyte glutathione peroxidase activity and plasma selenium concentrations in patients with phenylketonuria (PKU) or hyperphenylalaninemia (HPA) and in control subjects. The PKU patients had significantly lower glutathione peroxidase activity than did both the HPA patients (P < 0.05) and the control subjects (P < 0.001) [Dunn’s procedure for all multiple (pairwise) comparisons]. n = 24 PKU patients, 10 HPA patients, and 42 control subjects.
Free radical attack on uric acid results, among other things, in the formation of allantoin, which is excreted in urine (22). Although uric acid concentrations were not significantly different between groups, a positive correlation of uric acid with plasma selenium was found. Both the decreasing purine intake with increasing dietary protein restriction and an increased ROS attack on uric acid in a selenium-deficient state would explain this observation.

The degree of selenium deficiency in plasma was strongly correlated with the reduction in glutathione peroxidase activity in the erythrocytes, which represent an intracellular compartment of the body. Erythrocytes are for several reasons ideal model cells: only the selenium-dependent glutathione peroxidase is expressed in these cells (23) and they are by nature highly susceptible to oxidative stress because their membranes are rich in polyunsaturated fatty acids and because the cellular contents of oxygen and iron are high. Furthermore, selenium has a higher affinity for erythrocyte glutathione peroxidase than for plasma glutathione peroxidase (24). PKU patients had lower glutathione peroxidase activity than did both HPA patients and control subjects, which was not compensated for by higher glutathione transferase activity. HPA patients did not have lower glutathione peroxidase activity than did control subjects, even though selenium concentrations were lower in the patients. We assume that the lower selenium concentrations of the HPA patients were already sufficient for full expression of glutathione peroxidase activity.

GSH has an important protective function against the attack of ROS, directly and as the substrate of glutathione peroxidase (25). A decrease in intracellular GSH may be an early indication of the presence of oxidative stress. In fact, the GSH values of the PKU patients were lower than those in control subjects and were positively correlated with selenium deficiency. Because glutathione reductase activities were normal, GSH reduction was likely due to ROS stress. Both the lower glutathione peroxidase activity and the lower SOD activity in the patients, resulting in lower hydrogen peroxide scavenging and superoxide detoxification, may have contributed to the lower GSH concentrations in the patients than in the control subjects.

Thyroid hormone status

The selenoenzyme thyroxyde deiodinase converts T₄ to T₃ and rT₃ to T₃ in peripheral tissues. FT₄ and FT₃ represent the directly available functional part from the plasma pools of these hormones. Low plasma selenium concentrations had a moderate effect on the free thyroid hormones, much less than expected from the effects on erythrocyte glutathione peroxidase. The inverse correlations of FT₄ and rT₃ with selenium and the tendency toward a positive association of FT₃ with selenium supported the idea that the low selenium state in the PKU patients impaired the activity of thyroxine deiodinase. FT₄ concentrations were clearly elevated in PKU patients. However, because those of FT₃ were elevated as well, the ratio of FT₄ to FT₃ was not significantly different from that in the control subjects. The reasons for the higher free thyroid hormone concentrations in the patients are not known.

The associations of selenium with FT₄, FT₃, and rT₃ were less than that with glutathione peroxidase activity in the erythrocytes. Lower concentrations of selenium may be sufficient for optimal functioning of the enzyme (26), in agreement with the observation that in selenium-restricted animals thyroxine deiodinase activity was preferentially maintained and only decreased after depletion of glutathione peroxidase activity (27).

Selenium supplementation

Most organs and tissues, including erythrocytes and thyroid, as well as lung, heart, fibroblasts, brain gray matter, cerebellum, spleen, and pancreas, contain only the selenium-dependent glutathione peroxidase. Only a few human organs and tissues, including liver, skeletal muscle, and renal cortex, also contain a non-selenium-dependent glutathione peroxidase (23) that could supplement reduced selenium-dependent glutathione peroxidase activity. From the results in erythrocytes, reduced ROS defense could be postulated for organs that contain only the selenium-dependent glutathione peroxidase. Antioxidants often work in concert and one may partly substitute for another, but they are not fully interchangeable because they work in different compartments of the cell. Under normal conditions and with an adequate intake of vitamin E, ROS elimination in selenium-deficient patients may be sufficient; under stress conditions, however, it may fail. In premature infants with markedly reduced plasma selenium concentrations and erythrocyte glutathione peroxidase activity, a diet rich in polyunsaturated fatty acids and iron resulted in increased hemolysis (28), although serum α-tocopherol concentrations were normal. Our patients may have a similar risk of hemolysis when fed such a diet or when taking medications that exert a high oxidative stress. Thus, selenium substitution appears to be highly desirable for subjects consuming artificial, low-protein diets.

Daily selenium doses and the duration of selenium supplementation are controversial. According to the National Academy of Science, recommended dietary allowances for children of the ages in our study range from 20 to 50 μg Se/d (29). The best form of selenium to be administered is not known. Toxicity has been reported in association with intakes of >700 μg Se/d (30, 31). Selenomethionine is not an advisable form for supplementation because it may be nonselectively incorporated into body proteins instead of methionine, resulting in high tissue stores of selenium with potentially toxic consequences (32). Calomme et al (33) observed that PKU patients supplemented with 1 μg Se·kg⁻¹·d⁻¹ as selenate or a selenocysteine-containing bacterial protein mixture had normal selenium and glutathione peroxidase concentrations in plasma after 14 and 18 wk, respectively, and that no accumulation occurred in plasma. Cellular and tissue concentrations of glutathione peroxidase and selenium after supplementation are not known: replenishment may take longer and may bear the adverse effect of accumulation if not stopped at the right time.
Measurements of glutathione peroxidase activity and selenium concentrations in erythrocytes, although these are nonnucleated cells, may be good indicators of selenium recovery in tissues.

REFERENCES