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Research Articles

Thyroid function parameters during a selenium repletion/depletion study in phenylketonuric subjects†

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Abstract. Phenylketonuric (PKU) subjects have a limited supply of selenium (Se) in their phenylalanine-restricted diet. A Se repletion (1 μg Se/kg/day)/depletion study was conducted in PKU children to determine the effect of Se on thyroid function parameters.

The initial plasma Se concentration (mean ± SD: 0.26 ± 0.12 μmol/L, $p < 0.00003$, $n = 10$) and glutathione peroxidase (GSH-Px) activity (140 ± 58 U/L, $p < 0.00003$, $n = 10$) were significantly lower compared to age-matched controls. After 14 weeks of supplementation, the plasma Se concentration (mean ± SD: 0.74 ± 0.20 μmol/L) normalized (normal range: 0.57–1.15 μmol/L, mean ± SD: 0.76 ± 0.13 μmol/L, $n = 32$) and remained stable thereafter during repletion. Plasma GSH-Px activity reached normal values after 18 weeks of supplementation (312 ± 57 U/L; normal range: 238–492 U/L, mean ± SD: 345 ± 54 U/L, $n = 32$) and increased significantly for up to eight weeks thereafter (332 ± 52 U/L). Individual and mean thyroid parameters were initially normal in all cases. The mean concentrations of plasma thyroxine (T_4: $p < 0.025$), free T_4 (FT_4: $p < 0.01$) and reverse triiodothyronine (rT_3: $p < 0.005$) decreased to 75% of their initial value within three weeks of Se supplementation and remained stable thereafter, within a normal physiological range during selenium supplementation. They increased back to their initial values three weeks (T_4: $p < 0.05$, FT_4: $p < 0.05$) and six weeks (rT_3: $p < 0.025$) respectively, after the end of the supplementation. In conclusion, Se supplementation modifies thyroid function parameters in Se-deficient PKU subjects most likely by an increase in activity of type I 5′-deiodinase (5′-DIase I).

Key words. Phenylketonuria; selenium; glutathione peroxidase; thyroid hormones; type I 5′-deiodinase.

Phenylketonuria (PKU) is an inherited metabolic disease affecting approximately one in ten thousand children. Profound intellectual impairment has been prevented by the introduction of a low phenylalanine diet beginning as soon as possible after birth. Cases are detected within a few weeks after birth by neonatal screening in most Western countries. The restriction of whole protein foods in the diet of treated patients can cause a low concentration of some trace elements such as selenium (Se)[1,2]. The selenium status of PKU patients is similar to that of children living in some areas of severe endemic selenium deficiency in China, where it has been associated with a cardiomyopathy called Keshan disease[3]. In occidental countries, a similar deficiency has been associated in total parenteral nutrition with a reversible myopathy[4,5], increased creatine kinase activity, depigmentation of hair and nails[6], and, in some cases, a lethal cardiomyopathy[7,8].

Until recently, correction of selenium deficiency in PKU subjects was not considered necessary[9]. No clinical evidence has been reported so far of a clear benefit of selenium supplementation on the long term evolution of this group of patients, despite their profound biochemical deficiency[1,2]. Only one case report describes the effective treatment of cardiac dysrhythmia with selenium supplementation in a PKU patient[10].

Selenium is incorporated in the protein moiety of several selenoenzymes as selenocysteine. Seleno-glutathione peroxidases (GSH-Px: plasmatic, cellular and phospholipid hydroperoxide peroxidase) detoxify hydrogen peroxide and organic peroxides. Both the selenium concentration and the GSH-Px activity in blood compartments of PKU subjects are significantly lower compared to normal subjects[11,12]. Recently another selenocysteine-containing enzyme, namely type I 5′-deiodinase, was characterized in mammals[13–18]. This enzyme converts the prohormone thyroxine (T_4 or 3′,5′,3,5-te-

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traiodothyronine) into the biologically active hormone triiodothyronine (T_3 or 3',3,5-triiodothyronine), and the metabolically inactive reverse triiodothyronine (rT_3 or 3',5',3-triiodothyronine) into diiodothyronine (3',3-di-T_2). Eighty percent of serum T_3 is derived by this pathway from T_4 in the liver.

In view of this essential role of selenium in thyroid hormone metabolism, a selenium supplementation/depletion study was conducted in PKU childern to determine the effect of selenium on thyroid function parameters. The pharmacokinetics of selenium in deficient subjects were investigated in order to document the dosage required to reach optimal enzyme activity.

Subjects and methods

Subjects. After parental informed written consent, ten PKU children 6 to 18 years old (mean ± SD: 12.0 ± 4.1 years), 4 females and 6 males, were included in the study. Phenylketonuria was detected during the national neonatal screening programme and the subjects were continuously kept on a low phenylalanine diet from a few days after birth. The subjects weighed 20–56 kg (mean ± SD: 38.2 ± 13.3 kg). None received Se supplements for at least six months prior to the enrollment in the trial. The consumption of vitamins and minerals was strictly controlled throughout the complete study, to prevent selenium supplementation by commercial products. Blood was taken from children (mean age ± SD: 7.2 ± 4.0 y, n = 32) hospitalized in an orthopedic unit for minor ailments to determine control values for glutathione peroxidase activity and selenium concentration in plasma.

Study design. The protocol of the study was submitted and approved by the local Ethical Committee (Children Hospital, OCMW, Antwerp). Selenium was given for 46 weeks as tablets of sodium selenate (5 cases) or selenocysteine-containing bacterial protein mixture[19,20] (5 cases) at the dosage of 1 µg/kg/day in each subject. The tablets were prepared in the pharmacy of the hospital and were delivered to the subjects each month. The children were examined at home at various intervals during both the repletion (46 weeks) and the depletion (32 weeks) phase (see 'Results' section). Urine and blood samples (Li-heparine, Vacutainer, Beckton Dickinson) were taken at each time interval with irregular schedule, between 8 a.m. and 7 p.m. Blood samples were centrifuged within 24 h; urine and plasma were aliquoted and kept frozen (−20 °C).

Analytical methods. The plasma selenium concentration was measured by electrothermal atomic absorption spectrometry equipped with longitudinal Zeeman-effect background detection (Perkin Elmer model 4100 ZL, ETAAS)[21]. Seronorm reference serum was purchased to validate the method (batch 10017, Nycomed and Co, Oslo, Norway). The Se content of the tablets was verified, prior to administration, by flow injection hydride generation atomic absorption spectrometry (Perkin Elmer model 3100 equipped with MHS-FIAS-200 flow injection system, HGAAS) after wet acid digestion[22]. The accuracy of this method was checked against bovine liver (BCR 185, Community Bureau of Reference, Brussels, Belgium). The mean analyzed values of the reference standards were found to be within the certified range for both applied methods (HGAAS: 5.55 ± 0.37 µmol/kg compared to the certified value of 5.65 ± 0.16 µmol/kg; ETAAS: 1.20 ± 0.06 µmol/L compared to the recommended value of 1.22 µmol/L and range: 1.17–1.29 µmol/L).

Plasma glutathione peroxidase activity was measured at each time interval according to the method of Paglia and Valentine[23] modified by Beutler[24] with hydrogen peroxide as the substrate. One unit is defined as 1 micromole of NADPH oxidized per minute.

Plasma thyroid hormones (T_4, T_3, rT_3), thyrotropin (TSH) and thyroxine binding globulin (TBG) were determined in one batch for all the samples between zero and ten weeks' supplementation and in another batch for the remaining samples (14–78 weeks). All the assays were carried out in duplicate. Commercial radioimmunoassay reagents (TSH, T_4 and T_3: Amersham, UK; rT_3: Serono, Italy; TBG: Biocode, Belgium) were used. Urinary iodide was measured with an automated method.

Plasma cholesterol, apoprotein A1, apoprotein B were measured when sufficient plasma was available (at 42 or 46 weeks and at 78 weeks) with commercial reagents (Boehringer-Mannheim, automatic analyzer: Hitachi 717).

Pharmacokinetics and biochemical compartment analysis. The individual data of plasma selenium concentrations obtained during supplementation and depletion phases were analyzed with a software programme (SIPHAR, Simed, Creteil, France) giving the pharmacokinetic parameters. The food supply of selenium was considered to remain stable during the different phases of the study. The baseline selenium concentration reflecting the intake of selenium, was subtracted from each selenium concentration obtained in the study.

During the supplementation phase, the principal parameter was the half-life to reach a steady state; the general formula (* = multiplied by) is given by:

$$\text{selenium concentration at the Nth dose} = \text{dose}^* \frac{1-(\frac{1}{2})^{N\varepsilon}}{1-(\frac{1}{2})^{\varepsilon}} \quad \text{(equation 1)}$$

N is the number of doses administered and ε is the extraction coefficient. In the case of a daily administration, $\varepsilon = 1/\text{half-life}$ ($t_{\frac{1}{2}}$). The selenium concentration reaches a plateau at steady state and is given by:

$$\text{selenium concentration at steady state} = \text{dose}/1-(\tfrac{1}{2})^{\varepsilon} \quad \text{(equation 2)}$$

The individual data obtained for the selenium concentration in the depletion phase were fitted to exponential curves with one, two or three terms. The exponential

Table. Baseline selenium status and thyroid parameters.

	PKU		Controls	
	x ± SD	range	x ± SD	range
Plasma selenium status				
Selenium (μmol/L)	0.26 ± 0.13[1]	0.09–0.50	0.76 ± 0.13[2]	0.57–1.14[2]
Glutathione peroxidase (U/L)	140 ± 58[3]	72–260	345 ± 54[2]	238–492[2]
Thyroid parameters				
Total thyroxine (nmol/L)	120.5 ± 24.5	65.3–154.4	–[4]	58–160[5]
Free thyroxine (pmol/L)	16.6 ± 2.7	12.0–20.7	–[4]	10.0–30.0[5]
Total triiodothyronine (nmol/L)	1.99 ± 0.25	1.54–2.35	–[4]	1.20–2.50[5]
Total reverse triiodothyronine (pmol/L)	340 ± 126	127–501	–[4]	130–540[5]
Thyrotropin (mU/L)	1.4 ± 1.2	0.3–3.9	–[4]	0.3–4.2[5]
Thyroxine-binding globuline (mg/L)	20.2 ± 3.4	14.6–24.6	–[4]	12.0–26.0[5]

[1]$p < 0.00003$ (Mann-Witney U, one-tailed); plasma selenium concentration of PKU versus age-matched controls.
[2]Reference values of Belgium children and adolescents (age: 7.2 ± 4.0 y, range: 4–18 y, n = 32) hospitalized in an orthopedic unit for minor ailments.
[3]$p < 0.00003$; plasma glutathione peroxidase activity of PKU versus age-matched controls.
[4]Not available; [5]literature-based normal range in children and adolescents.

curve with two terms (bi-compartment model) fitted the experimental data for both supplementation forms best. The activity of plasma GSH-Px and of 5'-DIase I, as reflected by the change in plasma T_4 or of plasma rT_3, was considered to be in a pool equilibrium with the plasma selenium concentration. The relationships in plasma between the selenium concentration and both the GPX activity and the change in thyroid parameters (T_4, rT_3) were analyzed visually (see 'Results') permitting a linear regression analysis of the data under a threshold value of selenium.

Statistical analysis. Statistical parameters are expressed as the range or the arithmetic mean ± SD in the text and table, and as arithmetic mean ± SEM in the figures. The statistical significance of the change in follow-up parameters was analyzed with a one-tailed Wilcoxon matched pairs signed ranks test. The differences of parameters with values of normal subjects were evaluated with a Mann-Witney U test (one-sided). The association between two continuous variables was investigated with the Pearson's correlation procedure (two-sided). A value of $p < 0.05$ was considered to be statistically significant.

Results

The baseline, biochemical parameters of the ten PKU subjects are summarized in the table. The growth in stature and intellectual development were normal. Initial thyroid function parameters were normal in all cases. Both the selenium concentration ($p < 0.00003$) and the glutathione peroxidase activity ($p < 0.00003$) in plasma were significantly lower compared to healthy Belgian children, indicating a low selenium status of these PKU subjects. Iodine intake was adequate in all cases, as reflected by a sufficient iodine concentration in the urine (mean ± SD: 167 ± 89 μg/g creatinine, range: 82–389 μg/g creatinine; lower limit of controls: 40 μg/g creatinine).

The selenium concentration (fig. 1) increased within three weeks of supplementation from (mean ± SD) 0.26 ± 0.12 μmol/L to 0.44 ± 0.11 μmol/L ($p < 0.005$) and increased until 11 weeks thereafter (14 weeks: 0.74 ± 0.20 μmol/L, 10 weeks vs 14 weeks: $p < 0.005$). The mean selenium concentration remained stable between 14 and 46 weeks. The calculated half-life (mean ± SEM) to attain the steady state was 32.2 ± 7.06 days, and the corresponding extraction coefficient ε (mean ± SEM) was 0.0310 ± 0.0068. The plotted curve fitted the experimental data, and the expected selenium concentration at steady state (0.76 μmol/L) was similar to the mean concentration of normal subjects (mean ± SD: 0.76 ± 0.13 μmol/L,

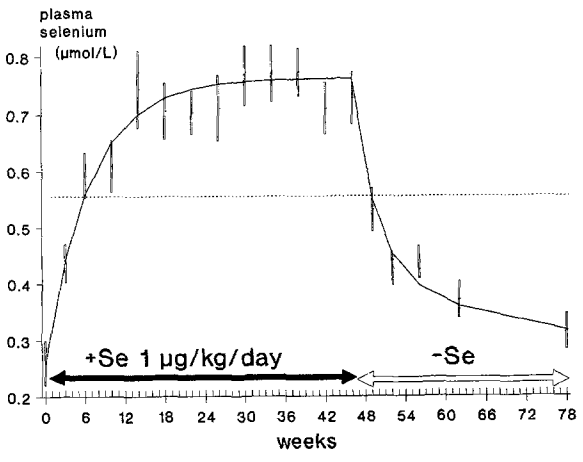

Figure 1. Effect of selenium supplementation (0–46 weeks) and depletion (46–78 weeks) on the plasma selenium concentration. Points represent mean values (n = 10) and vertical bars SEM. The plotted curve is given by equations 1 and 2 (supplementation, see 'Materials and methods') and by the following formula (depletion): Se conc. (t) = basal Se conc. + $0.3393\, e^{-0.0413t} + 0.1624\, e^{-0.0047t}$. The time (t) is expressed in days. The dotted line represents the lower limit of aged-matched controls (range: 0.57–1.47 μmol/L).

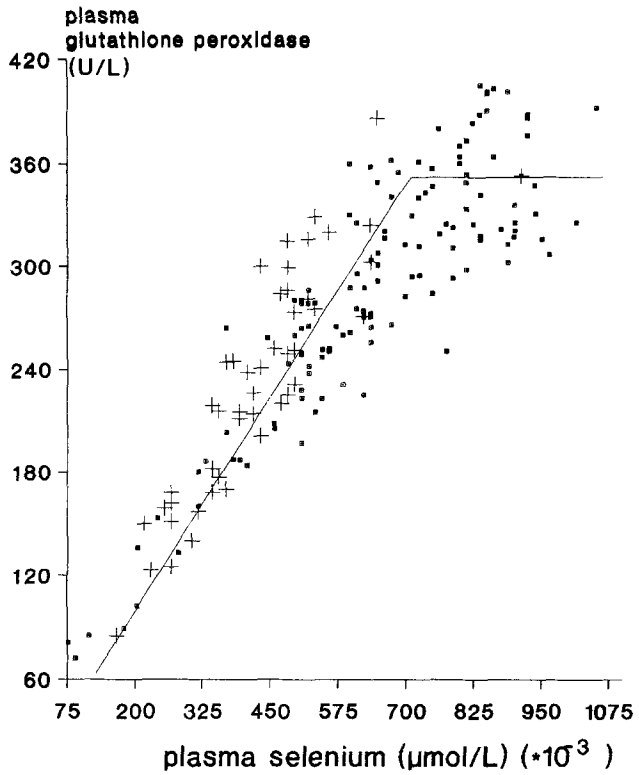

Figure 2. Relationship between selenium concentration and glutathione peroxidase (GSH-Px) activity in plasma. The dots represent the values during repletion (0–48 weeks) whereas the crosses represent the values obtained when the supplementation was stopped (46–78 weeks). The relationship under a threshold value of selenium (0.68 µmol/L) was linear (Pearson, $r = 0.93$, $p < 0.001$).

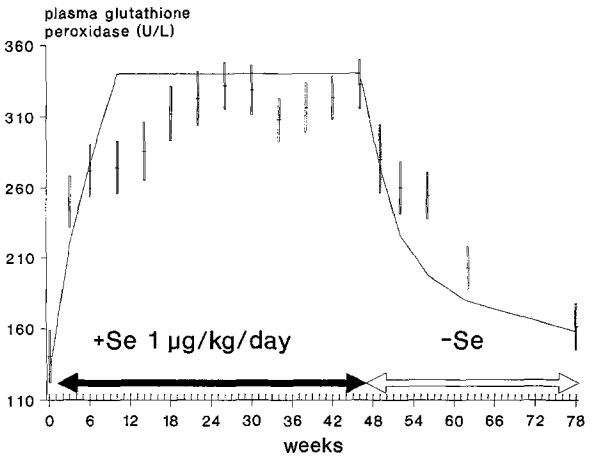

Figure 3. Effect of selenium supplementation (0–46 weeks) and depletion (46–78 weeks) on the plasma glutathione peroxidase (GSH-Px) activity. The dots represent mean values ($n = 10$) and vertical bars SEM. The plotted curve is given by the formula: plasma GSH-Px (U/L) = plasma selenium (µmol/L) *531.5 (equation 3, see 'Results').

$n = 32$). According to classical pharmacokinetic models, the steady state plateau is virtually reached after four half-lives (18 weeks), and this was indeed the case (observed value: 0.70 ± 0.15 µmol/L; expected value: 0.73 µmol/L). During the depletion phase, the experimental data fitted a bi-exponential model best. The general equation was:

$$\text{Se conc. (t)} = \text{basal Se conc.} + 0.3393\, e^{-0.0413t} + 0.1624\, e^{-0.0047t}$$

The basal selenium concentration is the mean selenium concentration observed when the subjects entered the study (0.26 µmol/L). The time (t) is expressed in days. The calculated curve fitted well the experimental data as is shown in figure 1. According to this model, the selenium elimination follows a bi-compartimental model, with an initial half-life of the rapid exchangeable pool of (mean ± SEM) 16.8 ± 2.8 days and a final half-life of the slow exchangeable pool of 147.7 ± 36.4 days. No significant differences were found between either supplemetation form regarding the calculated half-lives.

The relationship of the selenium concentration to the GSH-Px activity in plasma is shown in figure 2. Both the values obtained in the supplementation phase (first 46 weeks, dots) and in the depletion phase (46–78 weeks, crosses) are plotted. From visual inspection of the figure, it appears that the relation between plasma GSH-Px activity and selenium concentration corresponds, up to a plateau, to a linear regression model. The equation of the linear regression curve was calculated by taking into account the data under a threshold value of selenium (0.68 µmol/L) since the relationship appeared to be linear, by visual inspection of the figure, in this range of selenium concentration. A high correlation coefficient was found ($r = 0.93$, $p < 0.001$). The formula obtained by forcing the curve through the axis in this range is:

$$\text{plasma GSH-Px (U/L)} = \text{plasma selenium (µmol/L)} * 531.5 \qquad \text{(equation 3)}$$

The mean GSH-Px activity calculated for selenium concentrations above 0.76 µmol/L was 352 U/L, which is similar to the mean value of normal subjects (mean ± SD: 345 ± 54 U/L). Selenium concentrations between 0.68 and 0.76 µmol/L were not taken into account neither for the regression analysis nor for the plateau analysis since the curve has an inflexion point somewhere in between these values.

Initially, the plasma GSH-Px activity (fig. 3) was very low (mean ± SD: 140 ± 58 U/L, $p < 0.00003$, normal range: 238–492 U/L, mean ± SD: 345 ± 54 U/L) but increased significantly within three weeks of selenium supplementation (250 U/L, $p < 0.001$). It was found that plasma GSH-Px activity between 18 and 46 weeks' supplementation was not significantly different from normal subjects. However, the plasma GSH-Px activity at 18 weeks (312 ± 57 U/L) increased further up to 26 weeks' supplementation (332 ± 49 U/L, 18 weeks versus

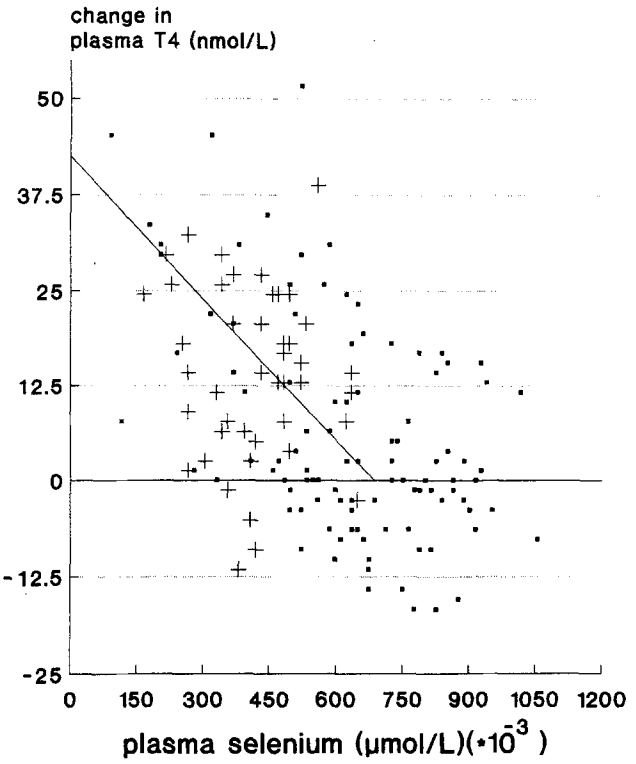

Figure 4. Relationship between the change in total thyroxine (T_4) and selenium concentration in plasma. The dots represent the values during repletion (first 46 weeks); the crosses represent the values obtained when selenium supplementation was stopped (46–78 weeks). The change in T_4 is defined as the difference between plasma T_4 at any time with the mean plasma T_4 measured when plasma selenium was above 0.76 µmol/L. The relationship under a threshold value of selenium (0.68 µmol/L) was linear (Pearson, $r = 0.47$, $p < 0.001$).

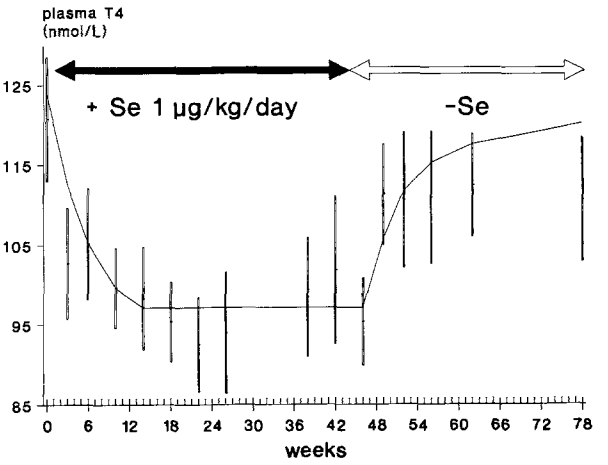

Figure 5. Effect of selenium supplementation (0–46 weeks) and depletion (46–78 weeks) on the plasma total thyroxine (T_4) concentration. The dots represent mean values ($n = 10$) and vertical bars SEM. The plotted curve is given by: the change in plasma T_4 (nmol/L) = 42.6 nmol/L – plasma selenium (µmol/L) *62.12 (equation 4, see 'Results').

26 weeks: $p < 0.025$) and remained stable thereafter (26–46 weeks). During selenium depletion (46–78 weeks), the plasma GSH-Px activity decreased significantly within three weeks (week 49: 280 ± 68 U/L, $p < 0.005$), and continued to decrease to a value close to the initial GSH-Px activity (week 78: 161 ± 48 U/L). The above formula (equation 3) was applied to the mean selenium concentrations, and the expected plasma GSH-Px activity (plotted curve) fitted the experimental data between the periods zero to six weeks and 46–78 weeks. Between 10 and 46 weeks, the calculated values deviated from the experimental data: the plasma GSH-Px activity increased significantly up to 26 weeks' supplementation while plasma selenium remained stable between 14 and 46 weeks.

The changes in plasma T_4 and rT_3 during selenium supplementation and depletion were used as parameters of variation in 5'-deiodinase I activity. The relation between the change in plasma T_4 and the plasma selenium concentration is shown in figure 4. The dots represent the values measured during repletion (first 46 weeks); the crosses represent the values obtained when selenium supplementation was stopped (46–78 weeks). The change in these parameters is defined as the difference between plasma T_4 or rT_3 at any time with the mean plasma T_4 or rT_3 given when plasma selenium was above 0.76 µmol/L (i.e. when the selenium concentration was in a steady state and when the 5'-DIase I activity is expected to be optimal). The correlation between the change in plasma T_4 with the plasma selenium concentration was lower ($r = 0.47$, $p < 0.001$) compared to that of plasma GSH-Px with plasma selenium ($r = 0.93$, $p < 0.001$). From visual inspection of the figure, it seems that the relationship between plasma T_4 and the selenium concentration fits a linear regression model up to a plateau. The equation of the linear regression curve was calculated by considering the data under the threshold value of selenium (0.68 µmol/L) since the relationship seemed to be linear, by visual inspection of the figure, in this range of selenium concentration. Within this range, the formula is:

change in plasma T_4 (nmol/L) = 42.6 nmol/L

– plasma selenium (µmol/L) *62.12 (equation 4)

The mean, calculated, T_4 concentration was maintained at 42.5 nmol/L for selenium levels above 0.76 µmol/L. Selenium concentrations between 0.68 and 0.76 µmol/L were not taken into account neither for the regression analysis nor for the plateau analysis, since the curve has an inflexion point somewhere in between these values. Initially, the plasma T_4 (fig. 5) was normal (120.4 ± 24.5 nmol/L; normal range: 57.9–154.4 nmol/L) but decreased significantly within three weeks of selenium supplementation (102.4 ± 22.0 nmol/L, $p < 0.001$), and continued to decrease slightly (NS) to a narrow range between 92.3 ± 17.6 nmol/L at 22 weeks and 101.5 ± 17.8 nmol/L at 42 weeks. During selenium de-

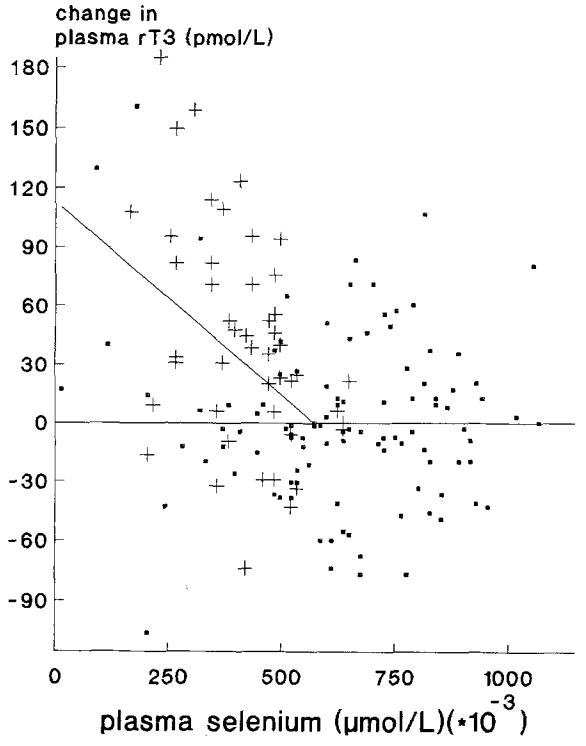

Figure 6. Relationship between the change in total reverse triiodothyronine (rT_3) and selenium concentration in plasma. The dots represent the values during repletion (first 46 weeks); the crosses represent the values obtained when selenium supplementation was stopped (46–78 weeks). The change in rT_3 is defined as the difference between plasma rT_3 at any time with the mean plasma rT_3 measured when plasma selenium was above 0.76 µmol/L. The relationship under a threshold value of selenium (0.68 µmol/L) was linear (Pearson, $r = 0.53$, $p < 0.001$).

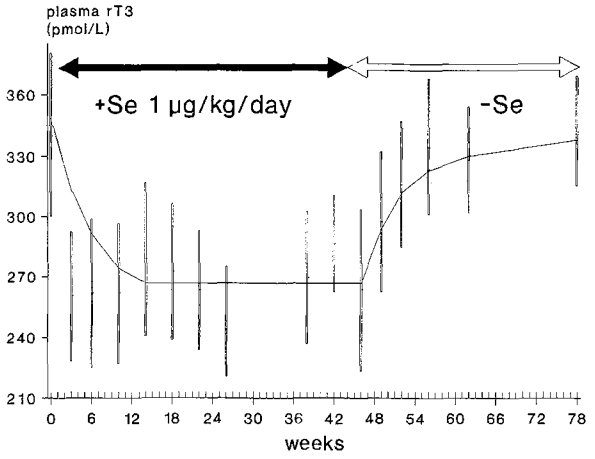

Figure 7. Effect of selenium supplementation (0–46 weeks) and depletion (46–78 weeks) on plasma total reverse triiodothyronine (rT_3) concentration. The dots represent mean values ($n = 10$) and vertical bars SEM. The plotted curve is given by: change in plasma rT_3 (pmol/L) = 110.9 pmol/L – plasma selenium (µmol/L) *0.242 (equation 5, see 'Results').

pletion the plasma T_4 concentration increased significantly within three weeks (49 weeks: 111.1 ± 17.8 nmol/L, $p < 0.05$) and remained stable until 78 weeks. The above formula (equation 4) was applied to the mean selenium concentrations, and the calculated plasma T_4 (plotted curve) fitted the experimental data from 0 to 78 weeks.

The change in plasma rT_3 during selenium supplementation and depletion is shown in figure 6. The dots represent the relationship of these parameters during repletion (first 46 weeks); the crosses represent the relationship of these parameters after selenium supplementation was ceased (46–78 weeks). The strength of association of the change in plasma rT_3 with plasma selenium concentration was significant ($r = 0.53$, $p < 0.001$) but the level of association was lower compared to the GSH-Px activity with the selenium concentration in plasma ($r = 0.93$, $p < 0.001$). Visual inspection of the figure suggests that the relationship between plasma T_4 with the selenium concentration fits a linear regression model up to a plateau. The equation of the linear regression curve was calculated considering the data under the threshold value of selenium (0.68 µmol/L) since the relationship seemed to be linear, after visual inspection of the figure, in this range of selenium concentration. Within this range, the change in plasma rT_3 is given by:

change in plasma rT_3 (pmol/L) = 110.9 pmol/L

– plasma selenium (µmol/L) *0.242 (equation 5)

Selenium concentrations between 0.68 and 0.76 µmol/L were not taken into account neither for the regression analysis nor for the plateau analysis, since the curve has an inflexion point somewhere in between these values. The initial plasma rT_3 concentration (fig. 7) was within a normal range (340 ± 126 pmol/L; normal range: 130–540 pmol/L) but decreased significantly within three weeks of selenium supplementation (259 ± 101 pmol/L, $p < 0.005$), and continued to decrease slightly (NS) to a narrow range between 242 ± 83 pmol/L at 26 weeks and 285 ± 72 pmol/L at 42 weeks. During selenium depletion, the plasma rT_3 concentration increased significantly within 6 weeks (52 weeks: 321 ± 92 pmol/L, $p < 0.025$), and increased slightly (NS) thereafter until 78 weeks (341 ± 80 pmol/L). The above formula (equation 5) was applied to the mean selenium concentrations, and the expected plasma rT_3 (plotted curve) fitted the experimental data from time 0 to 78 weeks.

Thyroid hormones are known to regulate the cholesterol level, mainly by regulating the density of LDL-receptors. The cholesterol level and the apoprotein B level (associated to the LDL-receptor) are good indices of thyroid status in liver at the cellular level. Sufficient plasma samples were available at 46 weeks' supplementation (when plasma selenium was in a steady state) and after 32 weeks' selenium depletion (78 weeks) to measure these parameters. All three lipid parameters were within the normal range at both times, and the mean values did not change significantly (46 weeks: choles-

terol: 3.53 ± 0.44 mmol/L; apoprotein A1: 1.128 ± 0.110 g/L; apoprotein B: 0.411 ± 0.061 g/L; 78 weeks: cholesterol: 3.53 ± 0.37 mmol/L; apoprotein A1: 1.107 ± 0.104 g/L; apoprotein B: 0.404 ± 0.043 g/L).

Discussion

The major source of selenium in the normal diet is protein. Several studies report that the selenium intake of treated PKU patients is lower than the safe and adequate daily intake[1,2] due to the phenylalanine restriction in their diet. The low intake of selenium-containing food proteins results in a low selenium status. The selenium intake of five PKU subjects (age: 7–18 years, mean ± SD: 10.8 ± 4.2 years) included in the present study was previously reported[25]. It was found that their intake was (mean ± SD: 7.8 ± 2.0 µg/day) more than six times lower compared to the 'estimated safe and adequate daily dietary intake for adolescents' (50–200 µg[26]). These observations were confirmed by the low plasma selenium status found for the ten PKU subjects in the present study.

The clinical importance of selenium in PKU remains to be documented. The recent characterization of 5'-DIase I as a selenoenzyme reopens the question concerning the clinical requirement of selenium for PKU.

Careful monitoring of selenium status, thyroid parameters and lipid parameters in PKU subjects during a selenium supplementation/depletion phase provides an opportunity to answer some questions.

1. What is the optimal level of selenium supplementation of PKU subjects?

According to our results, 1 µg/kg/day selenium as selenate or as selenocysteine-containing bacterial protein was sufficient to attain the mean plasma selenium concentration of age-matched controls (0.76 µmol/L). With the dosage regimen used in our protocol, the mean plasma GSH-Px activity at 26 weeks' supplementation was similar to the mean value of a normal population. Therefore, it seems that a higher selenium supply is not needed to reach an optimal enzyme activity. The daily dose of 1 µg/kg bodyweight which is comparable to the 'estimated safe and adequate daily dietary intake for adolescents' (0.87 µg/kg bodyweight[27]) was sufficient to correct the low plasma selenium status in PKU.

2. What are the metabolic characteristics of selenium in humans?

A slow depletion of selenium was shown in previous studies[28–30] with healthy volunteers taking between 105 and 162 days to decrease the selenium retention by a factor of 2. In agreement with these studies we found that the selenium pool can be represented by two compartments, one with a half-life of 17 days and another with a half-life of 148 days.

3. What is the relationship between the GSH-Px pool and the 5'-DIase I pool?

Previous experiments with animals suggested that selenium is used in a hierarchical manner in regulating the activity of GSH-Px and 5'-DIase I: the 5'-DIase I activity of restricted animals was perferentially maintained, and decreased only after depletion of GSH-Px activity[15]. This concept of hierarchy in selenium requirement does not explain the drop of thyroid hormones (T_4 and rT_3) within three weeks of stopping selenium supplementation in the present study. Therefore, our results suggest a multi-compartment model in which plasma selenium is exchanged with the enzymatic pool of GSH-Px and with the enzymatic pool of 5'-DIase I. The fact that, in this model, 5'-DIase I activity reaches a plateau earlier than the GSH-Px activity, could be explained by the difference in capacities of the two pools (GSH-Px pool capacity > 5'-DIase I activity). While the capacities of both enzymatic pools are expected to be significantly different, the affinity of both enzymes for selenium would be similar as the plasma selenium threshold values for maximal activity are very close. The conclusions of animal experiments support this hypothesis[31]. It was found that the nutritional selenium requirement of 5'-DIase was lower compared to that of GSH-Px: the adequate selenium concentration in the diet to attain optimal 5'-DIase-activity was 0.05 mg Se/kg whereas the GSH-Px activity with the same diet was only 50% of the optimal value.

4. Is the thyroid function normal in PKU children?

As selenium is essential for the conversion of the prohormone T_4 to the metabolically active hormone T_3 in peripheral tissues, it was important to determine whether there is evidence of thyroid hormone insufficiency in some metabolic pathways. Lipid metabolism is known to be finely regulated by the thyroid hormone levels, and cholesterol is increased in hypothyroidism mainly as a result of a decrease of LDL-receptors in the liver. The normal and stable lipid parameters before and after selenium depletion suggest that the intracellular level of T_3 concentration did not change significantly, at least in the metabolic pathways involving lipid metabolism. It can be concluded that the moderate increase of plasma T_4 associated with selenium depletion was sufficient to maintain an adequate level of T_3 not only in the plasma, but also at the cellular level (at least, as far as lipid metabolism is involved). Frank hypothyroidism at the tissue level in PKU children is excluded by the fact that their physical growth is normal. The conversion of T_4 to T_3 at the central level (brain and hypophysis), is dependent on another enzyme (5'-deiodinase II) which is known to be selenium-independent[18,32]. The absence of hypothyroidism at that level is demonstrated by the fact that PKU children have normal plasma TSH concentrations and normal

intellectual and psychomotor development (as long as their low protein diet is carefully respected). In fact, in another study normal plasma concentrations of TSH and T_3 were reported for PKU although the T_4 concentration was found to be significantly higher compared to controls[33].

The moderate increase of plasma T_4, FT_4 and rT_3 with selenium deficiency is in agreement with the recent finding that 5'-DIase I is the second characterized selenium-dependent enzyme in mammals. This enzyme converts T_4 to T_3 and rT_3 to T_2 in peripheral tissues. Selenium in vitro is indispensable for the activity of 5'-DIase I, and the enzyme activity in the absence of selenium is only 10%[34] that of the natural enzyme. While the effect of selenium on thyroid hormone metabolism is firmly documented in our data, it was found that the association of selenium with concentrations of thyroid hormones in plasma is much weaker than compared to selenium with GSH-Px. Apparently, the individual variations of thyroid hormones with selenium status are much more important than those of GSH-Px with selenium status. Selenium modulates thyroid hormone metabolism without being sufficient in itself to involve pathological changes even at very low selenium levels. Even when selenium concentration is extrapolated to a zero value, the maximal expected T_4 increased would be 42.6 nmol/L. Of course, our analysis is valid only if the linear regression applied to our data is still adequate for selenium levels close to zero.

The moderate increase of plasma T_4 in selenium-deficient subjects could be associated with a decrease of plasma TSH, as T_4 regulates TSH in a feedback loop. This was not observed but we do not believe that it was due to a methodological bias since we used an ultra-sensitive TSH assay (limit of sensitivity: 0.05 mU/L).

Conclusion

In conclusion, selenium supplementation modulates thyroid hormone metabolism in PKU children. The moderate increase of plasma T_4 in selenium-deficient children is compatible with an euthyroid state for the parameters analyzed (intellectal development, physical growth, lipid metabolism). Nevertheless, the lack of toxicity of selenium supplementation and the possible other mechanisms of action of this essential element, which remain to be documented in humans, suggest that selenium supplementation should be seriously considered for PKU children.

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