Effect of selenium deficiency on tissue taurine concentration and urinary taurine excretion in the rat

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The purpose of this study was to determine the effect of selenium deficiency on tissue taurine levels and urinary taurine excretion. Weanling male Sprague–Dawley rats were fed selenium-deficient or selenium-adequate diets for 20 weeks. As selenium deficiency developed, urinary taurine excretion increased in selenium-deficient rats compared to controls. At 12 weeks, the selenium-deficient rats excreted 1.7-fold more taurine than control rats. At the same time plasma glutathione peroxidase was 1.2% of control and plasma glutathione was 226% of control. At 20 weeks, renal taurine was decreased but renal glutathione was increased in selenium-deficient rats compared to controls. Feeding the experimental diet for 6 weeks without methionine supplementation caused a fall in urinary taurine excretion. However, there was no difference between selenium-deficient and control rats. These results indicate that selenium deficiency affects renal handling of taurine in the rat when dietary sulfur amino acids are not restricted.

Keywords: taurine; selenium deficiency; kidney; urine; glutathione; cysteine

Introduction

Taurine is a nonprotein sulfur amino acid that is derived from cysteine but is more abundant than its precursor. The only firmly established biochemical function of taurine is conjugation with bile acids in the liver.[1] The high levels of taurine in heart, muscle, brain, retina, kidney, and other organs suggest that it has other biological roles. Possibilities for function(s) of the compound include neurotransmission and neuromodulation in the brain and retina,[2,3] membrane stabilization,[3,4] osmoregulation,[1,5] and oxidant defense.[3]

Selenium deficiency affects sulfur amino acid metabolism in the rat. It causes an increased rate of glutathione synthesis in rat liver[6] which is associated with a decreased hepatic cysteine level. The increased synthesis of glutathione in the selenium-deficient liver is accompanied by an increased release of the compound into the circulation while hepatic glutathione concentration remains unchanged.

Plasma glutathione levels are elevated in the selenium-deficient rat as a result of the increased release of glutathione from the liver.[6] Plasma glutathione has a very short half-life[7] and it has been suggested that the kidney is largely responsible for its removal.[8] In the kidney, glutathione is degraded by γ-glutamyltranspeptidase and cysteinylglycine dipeptidase to its constituent amino acids[9] or taken up intact.[10,11] The selenium-deficient kidney has been shown to remove an increased percentage of plasma glutathione compared to the control kidney.[7] Thus, selenium deficiency affects glutathione metabolism in the liver, blood, and kidney.

Because taurine is derived from cysteine, changes in sulfur amino acid metabolism that affect cysteine have the potential to affect taurine levels. Selenium deficiency affects sulfur amino acid metabolism and this study was undertaken to determine the effect of

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selenium deficiency on taurine metabolism. Tissue taurine levels and urinary excretion were measured in selenium-deficient rats and compared with those measured in control rats.

Materials and methods

Animal care and diets

Male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN, USA) were fed a selenium-deficient or control diet from weaning. The Torula yeast-based diet[12] was supplemented with 0.3% *d,l*-methionine and 100 IU vitamin E/kg as all-rac-α-tocopheryl acetate (ordered from ICN Nutritional Biochemicals, Cleveland, OH, USA, as DL-α-tocopherol acetate). The control diet was supplemented with 0.5 mg selenium/kg as Na_2SeO_4. Selenium content of the unsupplemented diet was previously found to contain 0.008 μg selenium/kg diet.[17] The rats were housed in hanging wire-bottomed cages in a temperature-controlled room with a 12-h light/dark cycle.

Development of selenium deficiency

Weanling rats were randomized into two groups and fed selenium-deficient diet or control diet. Six rats from each group were designated for urinary taurine determination. These rats were weighed weekly and 24-h urine collections were made biweekly for 12 weeks. At 3, 6, 12, and 20 weeks, six rats from each diet group were anesthetized with pentobarbital (65 mg/kg). Blood was taken from the aorta, treated with disodium EDTA (1 mg/mL) and centrifuged immediately. The liver, kidneys, heart, brain, testis, and a portion of the right quadriceps muscle were removed. Plasma was separated from the red blood cells and plasma glutathione was measured as previously described.[7] The remainder of the plasma was used for measurement of taurine concentration and glutathione peroxidase activity. Tissues were weighed prior to being homogenized in water. A 1-g portion of liver was homogenized. The left kidney was homogenized in water for taurine determination and the right kidney was homogenized in 10% trichloroacetic acid for glutathione determination. The homogenates were centrifuged at 1500*g* for 15 min. The supernatants were frozen at −20°C for taurine measurement at a later time (usually within 2 weeks). The acid supernatant from the kidney was assayed on the same day for glutathione content. In addition an aliquot of the heart supernatant was acidified and the glutathione content was measured.

An additional 12 rats were fed control diet or selenium-deficient diet that was also deficient in sulfur amino acids (i.e., the supplemental methionine was omitted from the diet). These rats were weighed weekly. At 6 weeks, following urine collection, the rats were anesthetized and taurine levels were measured in plasma, liver, kidney, heart, brain, testis, and muscle in the manner described above.

Assays

Taurine was measured using a modification of a HPLC method reported by Porter *et al.*[13] Samples were prepared as described by Garvin.[14] An aliquot of urine, plasma, or tissue supernatant was placed in boiling water for 15 min to deproteinize the sample. Following centrifugation in a microfuge, 0.1 mL of the supernatant was applied to a mixed-ion exchange resin column and eluted with 2.0 mL of water. The eluant was collected and dried on a rotary evaporator. The dried sample was resuspended in 0.4–2.0 mL water. An aliquot of the aqueous sample containing 0.5–10 nmol taurine was derivatized with *o*-phthalaldehyde. γ-Glutamylglutamic acid was added as an internal standard prior to derivatization. The sample was injected onto a Spherisorb ODS-1 column (4.6 × 250 mm) and eluted with 5% CH_3OH:95% H_2O at a flow rate of 1 mL/min. The *o*-phthalaldehyde derivatives were detected at 340 nm. Recovery of taurine (200–400 nmol) added to urine samples was 94 ± 8% (n = 14) for selenium-deficient urine and 92 ± 8% (n = 19) for control urine.

Urinary sulfate was measured colorimetrically with barium chloranilate.[15] Recovery of sulfate (4–20 nmol) added to control urine was 116 ± 10% (n = 9).

Glutathione was measured in plasma and acidified tissue supernatants[7] as described previously. Plasma glutathione peroxidase activity was measured using 0.25 mM H_2O_2 as the substrate in the coupled assay described previously.[16]

Chemicals

Taurine, glutathione, GSSG reductase (EC 1.6.4.2), 5,5′-dithiobis (2-nitrobenzoic acid), and γ-glutamylglutamic acid were purchased from Sigma Chemical Co., St. Louis, MO, USA. *o*-Phthalaldehyde and HPLC-grade CH_3OH were obtained from Fisher (Atlanta, GA, USA). All other chemicals were of reagent grade. Dowex 1 and 50 ion exchange resins (200–400 mesh) were purchased from Bio-Rad (Richmond, CA, USA). Spherisorb ODS-1 HPLC column was obtained from ANSPEC (Ann Arbor, MI, USA).

Statistical analysis

Data were analyzed with Statview SE+ Graphics (Abacus Concepts, Berkeley, CA, USA). Student's unpaired t test was utilized to determine differences between control and selenium-deficient values, with $p < .05$ as the level of significance.

Results

The change in urinary taurine excretion with the development of selenium deficiency is shown in *Figure 1*. Selenium-deficient rats increased urinary taurine excretion between 2 and 4 weeks when compared with control rats. Taurine excretion was not significantly different than control at 6 weeks but was different at

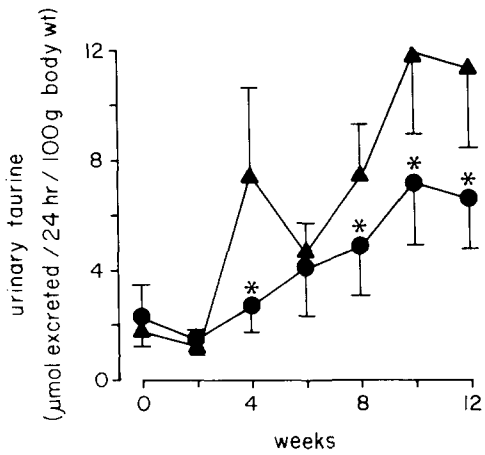

Figure 1 Change in urinary taurine excretion with the development of selenium deficiency. Rats were fed selenium-deficient (▲) or control diet (●). Control values marked with an asterisk are significantly different from selenium-deficient values measured at the same time ($p < .05$). Each point represents the average value for 6 rats and each bar represents one SD.

subsequent time points. Selenium-deficient plasma glutathione peroxidase activity was 8.5% of control (794 nmol NADPH oxidized/mL control plasma·min) at 3 weeks, verifying that the rats were moderately selenium-deficient. The glutathione peroxidase activity measured in selenium-deficient rat plasma continued to decrease with time (*Table 1*). It was 1.2% of control at 12 weeks. Thus, changes in urinary taurine excretion began when moderate selenium deficiency was achieved and these changes were maintained as the rats became severely selenium-deficient.

Plasma glutathione concentration was measured to determine if changes in it could be correlated with changes in taurine excretion. Previous dietary experiments have shown that plasma glutathione increased in selenium-deficient rats relative to control rats beginning at 3 weeks, and this difference became maximal at 5 weeks.[17] In this experiment plasma glutathione was measured initially and at 3, 6, 12, and 20 weeks (*Table 2*). By 3 weeks, selenium-deficient rat plasma contained 130% of the glutathione measured in control, indicating that changes in hepatic glutathione release had begun. Selenium-deficient plasma glutathione was twice that of control at 12 weeks. Thus, in this experiment, selenium-deficiency effects on urinary taurine excretion occurred in the same time frame as effects on plasma glutathione concentration and both were maximal by 12 weeks.

Table 3 shows the taurine concentration in several tissues at 20 weeks. The only tissue that showed any effect of selenium deficiency was the kidney. The changes in kidney taurine are shown in *Figure 2a*. By 12 weeks renal taurine concentration was significantly less in selenium-deficient kidney than in control. This difference was greater after 20 weeks.* Renal glutathione levels in selenium-deficient and control rats are shown in *Figure 2b*. The selenium-deficient kidney had

Table 1 Plasma GSH-Px activity[a]

Group	*n*	GSH-Px[b]
Selenium-deficient	6	10 ± 5
Control	5	2130 ± 260

[a] Values are means ± SD and were measured at 20 weeks. Selenium-deficient plasma GSH-Px activity was 8.5, 2.5, and 1.2% of control at 3,6, and 12 weeks, respectively.
[b] Units are nmol NADPH oxidized/(mL plasma·min). Selenium-deficient and control values are different ($p < .05$) by Student's *t* test.

Table 2 Plasma glutathione concentrations (nmol GSH equivalents/mL plasma)[a]

Week	Selenium-deficient	Control
0	—	12.8 ± 3.5
3	23.8 ± 3.9	18.3 ± 2.2
6	21.2 ± 3.2	16.2 ± 6.9
12	26.9 ± 2.7[b]	13.6 ± 2.2[b]
20	30.1 ± 3.7[c]	13.3 ± 1.6[c]

[a] Values are means ± SD for 6 selenium-deficient rats and 6 control rats, except 20-week value for which 5 control rats were used.
[b,c] Values with the same superscript are statistically different ($p < .05$) by Student's *t* test.

significantly more glutathione than control at 6 weeks and after.

Urinary sulfate excretion was not significantly different in selenium-deficient and control rats. At 12 weeks, selenium-deficient rats excreted 124 ± 26 mmol SO_4^{2-}/24 h/100 g body wt and control rats excreted 155 ± 44 mmol SO_4^{2-}/24 h/100 g body wt. The amount of sulfur excreted in the form of sulfate was 10–20 times the amount excreted as taurine. Thus, the difference found in taurine excretion between selenium-deficient and control rats could not be related to changes in sulfate excretion.

Methionine is usually added to the diet as a source of sulfur amino acids. The effect of selenium deficiency on urinary taurine excretion under conditions of limited sulfur amino acid supply was examined in rats that were fed a torula yeast-based selenium-deficient or control diet without added methionine. Methionine-deficient rats gained weight at the same rate regardless of selenium status, but their body weight gain was 40% less than that of methionine-adequate rats at 6 weeks. At 6 weeks, taurine was measured in a 24-h urine sample immediately prior to collection of tissues for determination of taurine concentration. The amount of taurine excreted in the urine

*Urinary excretion of taurine in selenium-deficient rats killed at 20 weeks was 150% of control. These rats were from the same dietary groups as those shown in *Figure 1*. However, the results shown in *Figure 1* were from the rats killed at 12 weeks for tissue taurine levels.

Table 3 Tissue taurine concentrations (μmol taurine/g wet wt)[a]

Tissue	Selenium-deficient	Control
Heart	10.1 ± 0.7	10.5 ± 0.5
Muscle	5.7 ± 0.8	5.2 ± 0.4
Kidney	3.7 ± 0.7[b]	5.4 ± 0.4[b]
Liver	0.4 ± 0.1	0.6 ± 0.2
Brain	1.4 ± 0.1	1.6 ± 0.2
Testis	1.3 ± 0.4	1.2 ± 0.1
Plasma	0.10 ± 0.01[c]	0.10 ± 0.01[c]

[a] Values are means ± SD for 6 selenium-deficient and 5 control rats. Measurements were made after feeding weanling rats the experimental diets for 20 weeks.
[b] Selenium-deficient and control values are different ($p < .05$) by Student's *t* test.
[c] Units are μmol taurine/mL.

was not affected by selenium status but was only 10–15% of the amount excreted in methionine-adequate rats (*Table 4*). Tissue taurine levels in methionine-deficient rats were significantly higher in the heart, liver, brain, and testis of rats maintained on selenium-deficient diet compared with rats fed control diet (*Table 4*). Muscle, kidney, and plasma taurine concentrations were not affected by selenium status in methionine-deficient rats.

Discussion

Taurine is an end product of sulfur amino acid metabolism resulting from the oxidation and decarboxylation of cysteine.[1] Cysteine is formed by hepatic transsulfuration of methionine[18] and is also made available by protein catabolism and by enzymatic degradation of glutathione.[9] Selenium deficiency affects sulfur amino

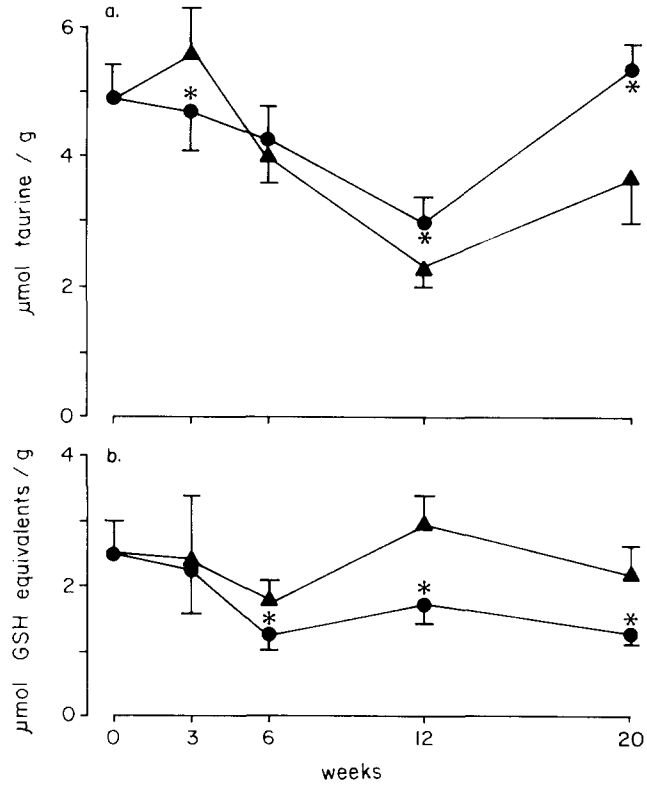

Figure 2 Changes in kidney taurine *(a)* and glutathione *(b)* concentrations with the development of selenium deficiency. Rats were fed selenium-deficient (▲) or control diet (●). Control values marked with an asterisk are significantly different from selenium-deficient values measured at the same time ($p < .05$). Each point represents the average value for 6 rats and each bar represents one SD.

Table 4 Organ taurine content in methionine-deficient and -adequate rats at 6 weeks[a]

Tissue	Diet	Methionine-deficient	Methionine-adequate
Heart	Selenium-deficient	10.6 ± 1.6[b]	16.1 ± 0.6
	Control	8.9 ± 0.8[b]	17.7 ± 3.4
Muscle	Selenium-deficient	3.3 ± 0.7	10.3 ± 1.8
	Control	2.7 ± 0.4	10.1 ± 1.8
Kidney	Selenium-deficient	1.5 ± 0.2	4.0 ± 0.4
	Control	1.3 ± 0.2	4.3 ± 0.5
Liver	Selenium-deficient	2.6 ± 0.5[c]	1.2 ± 0.2
	Control	1.9 ± 0.3[c]	1.2 ± 0.4
Brain	Selenium-deficient	3.1 ± 0.2[d]	4.3 ± 0.5
	Control	2.7 ± 0.3[d]	4.2 ± 0.7
Testis	Selenium-deficient	1.5 ± 0.1[e]	2.2 ± 0.3
	Control	1.2 ± 0.2[e]	2.0 ± 0.5
Plasma	Selenium-deficient	0.17 ± 0.03	0.18 ± 0.01
	Control	0.17 ± 0.03	0.16 ± 0.02
Urine	Selenium-deficient	0.57 ± 0.10	4.6 ± 1.1
	Control	0.61 ± 0.32	3.9 ± 1.8
Body wt	Selenium-deficient	146 ± 14	234 ± 8[f]
	Control	152 ± 11	255 ± 12[f]

[a] Values are means ± SD for 6 rats. Units are μmol taurine per g tissue, μmol taurine per mL plasma, or μmol taurine excreted per 24 hr per 100 g body wt. Body wt is given in grams.
[b–f] Values with the same superscript are statistically different ($p < .05$) by Students *t* test.

acid metabolism in the blood, liver, and kidney.[6,7] The experiments reported here describe the effect of selenium deficiency on taurine metabolism. Decreased renal taurine levels and increased urinary taurine excretion were demonstrated in the selenium-deficient rat.

One explanation of these findings is that selenium deficiency might cause a defect in renal taurine reabsorption. Urinary excretion of other compounds has been reported to be affected by selenium deficiency. Excretion of ketone bodies (acetoacetate and 3-hydroxy-butyrate) was reported to be approximately 10 times higher in selenium-deficient rats than in controls.[19] In addition, urinary excretion of sodium, potassium, urea, ammonium, and creatinine are increased two- to fourfold by selenium deficiency.[19] Thus, there may be defects in renal tubular transport in selenium deficiency.

An alternative explanation involves the increased renal blood flow measured in the selenium-deficient rat.[7] It was reported to be 1.7 times higher in selenium-deficient kidney than in control kidney.[7] The increased renal blood flow would be expected to result in increased glomerular filtration of plasma constituents. Thus, increased amounts of taurine would be filtered from the plasma into the urine. If the percentage of taurine reabsorbed was not increased then there would be a net increase in the amount of taurine excreted. This explanation does not address the question of decreased kidney taurine levels, however.

Taurine has been implicated in cardiac processes that involve calcium.[4,20–22] In isolated perfused chick hearts, taurine was effective in reducing the mechanical dysfunction resulting from calcium paradox.[20] Increased calcium levels that occur upon reperfusion of the heart with calcium were partially inhibited by pretreatment with taurine.[20] In cultured myocardial cells, taurine demonstrated a protective effect against irregular beating patterns seen when the cells were exposed to high or low extracellular calcium concentrations.[21] ATP-dependent Ca^{2+} transport is decreased in sarcolemma vesicles prepared from taurine-depleted hearts.[22] Thus, there is considerable evidence that taurine may be involved in calcium homeostasis. In addition it has been reported that rats with chemically depleted cardiac taurine levels have an increased rate of glycolysis with a simultaneous increased production of metabolic products from glucose.[23]

Another possibility is that taurine has an antioxidant function. Thomas and coworkers[24] reported that taurine protected erythrocytes from damage caused by the oxidative species formed by neutrophils. One implication of these experiments is that decreased taurine levels may predispose erythrocytes and other tissues to increased oxidant injury by neutrophils. Selenium has an antioxidant function and its absence results in increased sensitivity to oxidative challenges.[25] Thus, the relationship between taurine and selenium may be important in protecting against injury resulting from oxidative stresses.

It has been postulated that increased sensitivity to oxidant stress may be the precipitating factor in the development of Keshan disease, a cardiomyopathy found in selenium-deficient children in areas of China.[26] Pion *et al.*[27] reported a reversible cardiomyopathy associated with taurine deficiency in the cat. If cardiac taurine levels are decreased by selenium deficiency and if decreased cardiac taurine levels predispose the heart to oxidative injury, then an oxidant stress might lead to the cardiomyopathy found in Keshan disease. Further studies examining cardiac taurine levels after longer periods of selenium deficiency will be required to assess the possibility that cardiac levels of taurine decrease as reported for renal levels in this study.

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