Selenium bioavailability of soy-based diets in rats

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Bioavailability of selenium from soy grown with sodium selenite and sodium selenate was evaluated by the use of glutathione peroxidase activity regeneration in rats. Male weanling Sprague-Dawley rats were fed a selenium-deficient Torula yeast-based diet for 4 weeks followed by a 7-week repletion period during which animals were fed one of six experimental selenium repletion diets. Soybeans grown with selenite and selenate were processed into flour and used as the protein source in diets providing 50 and 100 ng/g selenium. Bioavailability was determined by liver and plasma glutathione peroxidase regeneration and tissue selenium repletion in response to Se in soy diets and compared with changes in response to Se in reference diets containing Torula yeast supplemented with sodium selenite. Results indicate that selenium from soy was as bioavailable as sodium selenite from the reference diet. In addition, selenium from soy grown with sodium selenite was as bioavailable to the rat as selenium from soy grown with sodium selenate. These data indicate that soy protein provided a bioavailable source of dietary selenium to rats and that the form of selenium available in plants did not influence selenium bioavailability of the resulting soy-based product.

Keywords: selenium; bioavailability; soybean; glutathione peroxidase

Introduction

Selenium (Se), an essential trace element, functions in the enzyme glutathione peroxidase (GSH-Px)[1] and may be involved in inhibiting certain forms of cancer.[2] The National Research Council (NRC) has established a recommended dietary allowance for Se of 55 μg for women and 70 μg for men.[3] An average balanced diet will provide this level of Se, however, individuals consuming a restricted diet, such as unsupplemented enteral feeding or infant formula, may not consume recommended amounts. Zabel et al.[4] found that, based on average intakes, several soy protein formulations did not supply the level of Se recommended by the NRC. When individuals consume a restricted diet source, it is essential that all recommended levels of nutrients be met.

Soybean protein is currently used in many restricted diet products such as infant formula and enteral feedings due to its high quality protein. However, one major disdavantage of using soy protein is its ability to decrease bioavailability of certain minerals. For example, zinc has a lower bioavailability in the presence of soy protein.[5] Other essential minerals such as iron, copper, and manganese may also be less bioavailable from soy than from animal protein sources. One of the factors in soy thought to inhibit mineral absorption is its high phytate content. Iron absorption in animals and humans is inhibited by phytate.[6,7] The absorption of selenium was decreased by phytate in humans when measured by balance studies.[8] This study does not specifically measure the effect of phytate on selenium absorption, but the effect of the use of soy protein in a diet on selenium utilization. Use of soy protein as a source of dietary Se is also complicated by the fact that the concentration of Se in soybeans is dependent on many factors, including chemical form and concentration of the mineral in soil. This study investigated the ability of a soy flour-based diet to provide biologically available Se to the rat and whether the form of Se taken up by the soybean plant had an effect on animal utilization of the mineral.

Methods and materials

Animals and diets

Male weanling Sprague-Dawley rats (Harlan Industries, Indianapolis, IN USA) were housed individually in hanging stainless-steel wire-mesh floored cages. Rats were provided with

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diet and deionized water ad libitum and were weighed weekly. Care and use of laboratory animals was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication 86-23). Room temperature was maintained at 22 ± 1° C with 10–15 air changes per hr on a 12-hr light/dark cycle. Rats were initially fed a Se-deficient Torula yeast basal diet (*Table 1*) for 4 weeks to bring them to a baseline low Se status before introduction of a repletion diet.

Soybean seeds (*Glycine max* L. Merr, 'Century 84') were germinated in either vermiculite or cellulose grow blocks. After 3 weeks of growth, seedlings were placed into four circulating hydroponic systems and provided with a modified Hoagland's nutrient solution.[9] The pH of the system was maintained between 5.5 and 6.0. Se was added to the nutrient solution as either sodium selenite or sodium selenate at concentrations of 2.5 and 25 ng/g.

Harvested experimental soybeans and locally grown (Purdue University, Agronomy Farm, West Lafayette, IN USA) low Se, Hack variety soybeans were processed into a lowfat flour. Soybeans were heat tempered, cracked, dehulled, and milled into a fine flour using a Model 4 Wiley mill. The flour was defatted with hexane and autoclaved to inactivate trypsin inhibitors following the procedure of Levine et al.[9]

Experimental diets were formulated to contain 50 ng/g and 100 ng/g Se. Reference diets were made with Torula yeast and supplemented with sodium selenite (REF-ITE). Soy-based diets were formulated with hydroponically grown sodium selenite (SOY-ITE) or sodium selenate (SOY-ATE) soy flour. Additional low-Se containing soy flour was used to provide the appropriate protein concentration to the soy diets. All experimental diets were nutritionally complete and provided 25% protein and 7.8% fat as shown in *Table 1*. The Se concentration by analysis of repletion diets is shown in *Table 2*.

Table 1 Composition of experimental diets[a]

Diet component	Depletion diet (g/100 g)[b]	Repletion diets (g/100 g)	
		Torula yeast	Soy
Torula yeast	49.7	49.7	
Soy flour			55.0
Sucrose	33.5	33.5	27.2
Fiber (Alphacel)[d,f]	5.0	5.0	5.0
Corn oil[g]	7.0	7.0	7.0
AIN mineral mix[d,h]	3.5	3.5	3.5
AIN vitamin mix[d]	1.0	1.0	1.0
Choline bitartrate[d]	0.2	0.2	0.2
DL-methionine[d]	0.1	0.1	0.1
Sodium selenite	—	+[i]	—

[a]All diets were formulated to contain 25% protein.
[b]Se deficient (less than 2 ng/g).
[c]Torula yeast contained 50.3% protein, 1.65% fat, and 4 ng/g Se.
[d]ICN Nutritional Chemicals, Cleveland, OH USA.
[e]Soy flour contained 45.5% protein, 2.45% fat, 56 ng/g Se (selenite), 39 ng/g Se (selenate). Hydroponically grown high-Se flour was mixed with low-Se locally grown flour to result in 50 and 100 ng Se/g repletion soy diets.
[f]Provided nonnutritive bulk.
[g]Mazola, purchased locally.
[h]Mineral mix without Se.
[i]Sodium selenite added to torula yeast repletion diets at levels of 50 ng/g and 100 ng/g Se.

Experimental protocol

At arrival, two rats were killed for the determination of initial Se status in liver and plasma. Four weeks after feeding rats the Se-deficient Torula yeast diet, three additional animals were killed to determine baseline Se status. Animals were then randomly assigned to six groups and fed one of the experimental diets. Three rats in each group were killed by CO_2 at 2, 4, 6, and 7 weeks of the repletion period. Chests of animals were surgically opened and blood was drawn from the left ventricle by cardiac puncture after injection of 0.25 mL anticoagulant (35 mmol/L citric acid, 75 mmol/L sodium citrate dihydrate, 140 mmol/L glucose). Approximately 3.0 mL of blood was drawn and placed into a tube containing 0.25 mL of anticoagulant on ice, then centrifuged at 550*g* at 4° C for 15 minutes. Plasma samples were divided into two portions. One portion was immediately assayed to determine plasma GSH-Px activity. The second portion was frozen at −20° C for later Se determination.

Intact livers were perfused with normal saline, rinsed with deionized water, blotted dry, weighed, and placed in four volumes of cold 0.15 M potassium chloride. All procedures were carried out on ice or in refrigerated instruments (0–4° C). Liver tissue was homogenized with a Polytron homogenizer (Brinkmann Industries, Westbury, NY USA) and centrifuged at 5000*g* for 15 minutes to remove mitochondria and connective tissue. The mitochondrial-free supernatant fraction was decanted and centrifuged (Model L5-50, Ti50 rotor, Beckman Instruments, Palo Alto, CA USA) at 100,000*g* for 1 hr. The microsomal supernatant fraction was decanted in two portions. One portion was immediately assayed to determine liver GSH-Px activity while the other portion was frozen at −20° C for later Se determination.

Analysis

GSH-Px activity in plasma and liver supernatant fractions was measured by the coupled method of Paglia and Valentine[10] with the modifications of Levander et al.[11] using 15 mmol/L t-butyl hydroperoxide as the substrate. Enzyme activity was expressed as units/mg protein (equivalent to μmoles NADPH oxidized/min/mg protein). Protein was measured by the method of Lowry et al.[12] using bovine serum albumin as a protein standard.

Se in plasma, liver supernatant, and diets was analyzed by gas chromatography using the method of McCarthy et al.[13] Se concentration of plasma and liver was expressed at μg Se per mg protein ([Se]/[prot]). Samples were analyzed on a Varian (Walnut Creek, CA USA) 3400 gas chromatograph and Model 8000 autosampler equipped with a ^{63}Ni electron-capture detector. Nitrogen was used as the carrier gas at a flow rate of 30 mL/min. A silanized glass column packed with 3% OV-225 on 100–120 mesh Supelcoport purchased from Supelco Inc. (Bellfonte, PA USA) was used for the separation of the O-diamine derivative of Se. A Shimadzu (Kyoto, Japan) Model C-R3A Chromatopac integrator was used to analyze chromatograms.

Statistical analysis

Data were analyzed using the Statistical Analysis System (Cary, NC USA).[14] Means were compared using a two-way analysis of variance and the Student Newman-Keuls test, using $P < 0.05$ as the level of significance.

Results

Upon arrival, mean baseline GSH-Px activity in liver and plasma was 0.1307 and 0.0178 units/mg protein,

Table 2 Selenium concentration of repletion diets

Dietary code	Dietary group	Dietary description	[Se][a](ng/g)
REF-ITE	50 ng/g	sodium selenite added to Torula yeast	53.3 ± 4.1
REF-ITE	100 ng/g	sodium selenite added to Torula yeast	104.4 ± 2.6
SOY-ITE	50 ng/g	soy hydroponically grown in sodium selenite	57.1 ± 5.3
SOY-ITE	100 ng/g	soy hydroponically grown in sodium selenite	80.8 ± 3.2
SOY-ATE	50 ng/g	soy hydroponically grown in sodium selenate	46.9 ± 2.8
SOY-ATE	100 ng/g	soy hydroponically grown in sodium selenate	91.5 ± 4.2

[a]Values are means ± SE ($n=5$). Selenium concentration of diets was determined by the gas chromatographic method of McCarthy et al.[10]

respectively. After four weeks on the Se-deficient diet, mean liver and plasma GSH-Px activities of rats were 0.0333 and 0.0011 units/mg protein representing a four-fold and 16-fold decrease, respectively, from mean baseline levels. Throughout the study, no significant differences in mean body weight or total food intake were found between rats fed any of the repletion diets, indicating that the type of protein source or Se concentration in the diet was not affecting the overall growth of the animals. GSH-Px activity in liver and plasma were measured throughout the experimental period and are shown in *Figures 1A and 1B*. GSH-Px activity in both liver and plasma increased over baseline levels throughout the experiment. When rats were fed 100 ng/g Se diets over the 7-week experimental period, liver GSH-Px levels increased at a greater rate than plasma GSH-Px. Tissue [Se]/[prot] in liver and plasma were measured throughout the experimental time period and are shown in *Figures 2A and 2B*. In addition, liver [Se]/[prot] levels also showed similar regeneration rates as liver GSH-Px versus plasma, increasing quickly over the 7-week repletion period.

GSH-Px activity and Se concentration levels in liver and plasma at the end of the experiment are shown in *Table 3*. Levels of GSH-Px and [Se]/[prot] in liver and plasma were higher when animals were

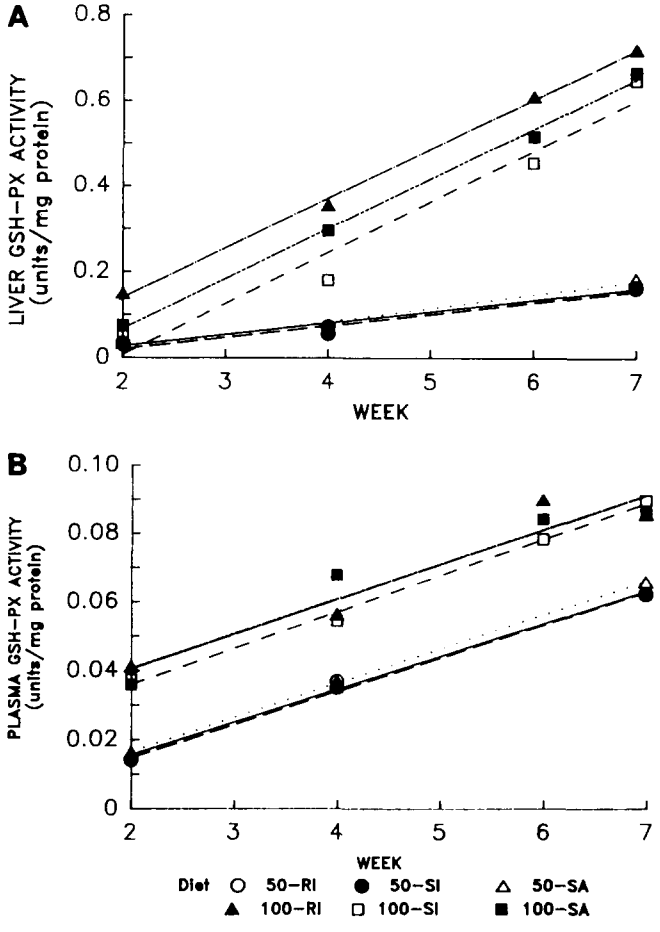

Figure 1 GSH-Px activity regeneration.

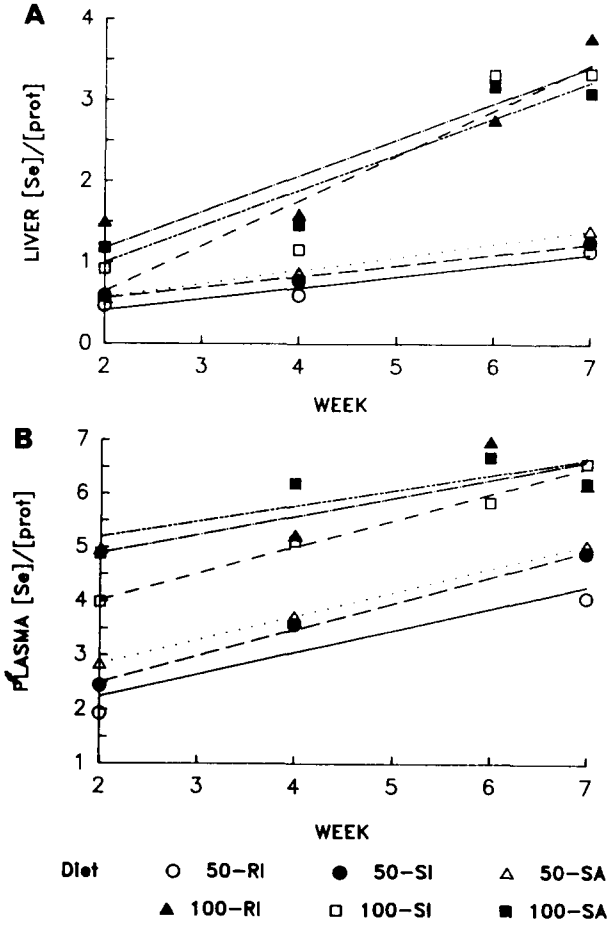

Figure 2 Tissue [Se]/[Prot] regeneration.

Table 3 Bioavailability parameter responses on week 7*†

Diet	GSH-Px Activity‡ Liver	GSH-Px Activity‡ Plasma	[Se] (μg/g) Liver	[Se] (μg/g) Plasma	[se]/[prot] Liver	[se]/[prot]§ Plasma
50-RI	0.1648[a] ± 0.0114	0.0622[a] ± 0.0013	0.0199[a] ± 0.0011	0.2643[a] ± 0.0152	1.141[a] ± 0.035	4.074[a] ± 0.230
50-SI	0.1624[a] ± 0.0170	0.0624[a] ± 0.0046	0.0212[a] ± 0.0005	0.3266[ab] ± 0.0395	1.253[a] ± 0.075	4.901[b] ± 0.402
50-SA	0.1851[a] ± 0.0413	0.0659[a] ± 0.0068	0.0214[a] ± 0.0021	0.2904[ab] ± 0.0103	1.403[a] ± 0.154	5.058[b] ± 0.240
100-RI	0.7189[b] ± 0.0622	0.0856[ab] ± 0.0045	0.0717[c] ± 0.0094	0.3644[b] ± 0.0149	3.776[b] ± 0.535	6.181[c] ± 0.181
100-SI	0.6482[b] ± 0.0499	0.0897[b] ± 0.0087	0.0512[b] ± 0.0076	0.3638[b] ± 0.0069	3.331[b] ± 0.069	6.578[c] ± 0.141
100-SA	0.6660[b] ± 0.0583	0.0859[ab] ± 0.0046	0.0532[b] ± 0.0008	0.3504[b] ± 0.0057	3.092[b] ± 0.271	6.215[c] ± 0.150

*Mean ± S.E.M. $n = 3$.
†Within columns, means followed by the same letter are not significantly different ($P \leq 0.05$).
‡GSH-Px activity = μmoles NADPH oxidized /min/ mg. protein.
§[Se]/[prot] = $\frac{\text{selenium content (μg/g) of sample}}{\text{protein content (μg) of sample}}$.

fed 100 ng/g diets than when they were fed 50 ng/g diets. However, only one significant difference was found at either the 50 or 100 ng/g Se diet level between the reference diet and the two test soy-based diets. At the end of the experimental 7-week period, plasma [Se]/[prot] levels for the 50 ppb Se-fed animals were significantly greater in animals fed either soy diet than in animals fed the reference diet. After 7 weeks, liver [Se] was greater in 100 ng/g Se reference diet-fed animals than either 100 ng/g Se soy-based diet-fed animals.

Discussion

Although Se from soy protein is well absorbed,[15,16] two previous studies evaluated the bioavailability of Se from soy as being mediocre. Cantor et al.[17] assessed the bioavailability of Se from soy using prevention of exudative diathesis (ED) in chicks. A point-slope regression equation was generated by plotting percentage protection against ED versus the log of dietary Se concentration. Se from soy isolate was calculated to be 59.8% bioavailable.

The use of disease prevention methods to estimate Se bioavailability has the limitation that one form of Se may prevent ED but may have no effect on reversing other biological manifestations of Se deficiency. Although Cantor et al.[17] used the disease prevention approach to estimate Se bioavailability, results may be more accurate than those determined by Gabrielsen and Opstvedt.[18] In their study, bioavailability of Se from solvent-extracted soybean meal was calculated to be 17.5%. Bioavailability was assessed in 1-day-old chicks using blood serum GSH-Px activity regeneration. A Se-deficient diet containing 40 ng/g Se was fed to chicks for 7 days. The short time period of depletion coupled with the high dietary Se content of the depletion diet may not have ensured Se deficiency. Graded levels of dietary Se were incorporated into a soy based diet containing approximately 30% soy and 25% Toprina (single-cell protein) and fed to chicks for only 9 days. This repletion period was too short to accurately determine Se bioavailability. In the same study, bioavailability of Se from selenomethionine was determined to be 78.3%, a value below previous estimates of selenomethionine bioavailability.[19,20] Thus, Se bioavailability in the Gabrielsen and Opstvedt[18] study may have been underestimated.

In this study, bioavailability was determined by the regeneration of GSH-Px activity and Se concentration in liver and plasma of Se-depleted rats in response to a reference diet and soy flour protein-based diets. Adequate baseline Se deficiency was initially produced in this study as indicated by the drop in GSH-Px activity, similarly observed in previous studies.[11,16] Results of liver and plasma GSH-Px activity and tissue Se regeneration suggest that there was no significant difference between the bioavailability of Se from soy as compared with the reference diet. The bioavailability of Se from soy grown with sodium selenite was not different in the rat than Se from soy grown with sodium selenate. Differences in the form of Se deposited in corn are known to occur depending on the form of Se available to the plant. Gissel-Nielsen[21] found that Se from selenite was associated with amino acids in corn phloem, while Se from selenate was translocated as selenate. The form of Se in wheat was reported as selenomethionine,[22] and selenomethione has been identified in soybean proteins.[23] There are other selenium-containing compounds in soy, and assumptions cannot be made on the availability of selenium from soy based on the availability of selenomethionine.

This study, along with two previous studies from this laboratory,[15,16] provide evidence that Se from soy is both well absorbed and as bioavailable as Se from sodium selenite salt using the GSH-Px assay. In addition, the form of Se available to soybeans did not influence Se bioavailability of the resulting soy-based product; however, caution must still be used when relying solely on soy sources for daily Se requirements due to regional variations in soy Se concentration. As Zabel et al.[4] observed, common unsupplemented soy-based infant and total enteral

formulas provided Se levels well below amounts recommended by the NRC. To increase Se intake of persons relying on low Se soy-based formula, supplementation of these products with Se is warranted.

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