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Selenium Bioavailability from Soy Protein Isolate and Tofu in Rats Fed a Torula Yeast-Based Diet

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Selenium (Se) is an essential nutrient, and soy is a major plant source of dietary protein to humans. The United States produces one-third of the world's soybeans, and the Se-rich Northern Plains produce a large share of the nation's soybeans. The present study used a rat model to determine the bioavailability of Se from a protein isolate and tofu (bean curd) prepared from a soybean cultivar we recently developed specifically for food grade markets. The soybean seeds contained 2.91 mg Se/kg. Male Sprague−Dawley rats were depleted of Se by feeding them a 30% Torula yeast-based diet containing 5 μg Se/kg; after 56 days, they were replenished of Se for an additional 50 days by feeding them the same diet supplemented with 20, 30, or 40 μg Se/kg from soy protein isolate or tofu. L-Selenomethionine (SeMet) was used as a reference. Selenium bioavailability was determined on the basis of the responses of Se-dependent enzyme activities and tissue Se contents, comparing those responses for each soy product to those for SeMet using a slope-ratio method. Dietary supplementation with the protein isolate or tofu resulted in dose-dependent increases in glutathione peroxidase activities in blood and liver and thioredoxin reductase activity in liver, as well as dose-dependent increases in the Se contents of plasma, liver, muscle, and kidneys. These responses indicated an overall bioavailability of approximately 97% for Se from both the protein isolate and tofu, relative to SeMet. These results demonstrate that Se from this soybean cultivar is highly bioavailable in this model and that high-Se soybeans can be good dietary sources of Se.

KEYWORDS: Selenium; selenium bioavailability; Torula yeast; soybean; rats

INTRODUCTION

Selenium (Se) is an essential nutrient for humans and animals, and its deficiency is related to the risk of Keshan disease, a cardiomyopathy primarily affecting children and young women (*1*), and white muscle disease (muscular dystrophy) in domestic livestock (*2*). An adequate Se intake is essential to human health, and the current recommended daily allowance for Se is 55 μg/day for both adult men and women in the United States (*3*). Selenium is an integral part of the active site of several enzymes, including glutathione peroxidase (GPX) (*4*) and thioredoxin reductase (TRR) (*5*), which are involved in detoxification, the oxidation/reduction reaction, and cellular metabolisms.

Soy has been a major plant source of dietary protein for humans for centuries. Consumption of soy foods is considered beneficial to heart health (*6*) and cancer prevention (*7*). The United States Food and Drug Administration authorized a health claim in 1999 that the consumption of soy protein may reduce the risk of heart disease (*8*). The United States produces approximately one-third of the world's soybeans, and the Northern Plains states are among the major soy producing states of the country. The Se contents of soybeans reflect the Se contents of the producing soils, which vary widely among the major soy producing states. In general, soils in the Northern Plains are adequate or high in Se (*9*) such that soybeans produced in this region tend to have greater Se contents than those produced elsewhere (*10*). Still, the nutritional value of the high-Se soybeans produced in this region has not been assessed.

We recently developed a soybean cultivar which is suitable for use in specialty food-grade markets for tofu and soy milk production. We used this cultivar to produce high-Se soybeans similar to those grown on high-Se soils by applying Se to the plants during soybean seed development. The purpose of the present study was to determine the bioavailability of Se from the protein isolate and tofu (bean curd) prepared from that soybean cultivar relative to that of Se from selenomethionine (SeMet), a reference standard. Soy protein isolate has wide applications in the food industry, and tofu is one of the most commonly consumed traditional soy foods.

MATERIALS AND METHODS

This study was approved by the Animal Use Committee of the U.S. Department of Agriculture, Agricultural Research Service,

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Table 1. Composition of the Basal Diet[a]

ingredient	g/kg
corn starch	427.4
Torula yeast	300.0
sucrose	100.0
soybean oil	70.0
cellulose	50.0
mineral mix[b]	35.0
AIN-93G vitamin mix	10.0
L-methionine	2.9
L-cystine	3.5
L-tryptophan	0.16
Choline bitartrate	1.0

[a] At 30% of the diet, Torula yeast (*15*) provides an adequate amount of all essential amino acids for rodents except for cystine (1.8 g/kg), methionine (2.4 g/kg), and tryptophan (1.84 g/kg) according to NRC recommendations for cystine (4.9 g/kg), methionine (4.9 g/kg), and tryptophan (2 g/kg) (*16*). Thus, these amino acids were added to the diet to meet the recommendations. At 30% of the diet, the yeast provides 0.9 g/kg choline, and we added an additional 0.4 g choline/kg (1.0 g choline bitartrate/kg) to the diet to meet that provided by the AIN-93G formulation (*14*). [b] Mineral mix contained calcium carbonate, anhydrous, 40.04% Ca, 555.26 g/kg; sodium chloride, 39.34% Na, 52.17 g/kg; sodium *meta*-silicate, 9 hydrate, 9.88% Si, 1.45 g/kg; chromium potassium sulfate, 12 hydrate, 10.42% Cr, 0.275 g/kg; copper carbonate, 57.47% Cu, 0.143 g/kg; boric acid, 17.5% B, 0.082 g/kg; sodium fluoride, 45.24% F, 0.064 g/kg; nickel carbonate, 45% Ni, 0.032 g/kg; lithium chloride, 16.38% Li, 0.017 g/kg; potassium iodate, 59.3% I, 0.010 g/kg; ammonium paramolybdate, 4 hydrate, 54.34% Mo, 0.008 g/kg; ammonium vanadate, 43.55% V, 0.007 g/kg; and powdered sucrose 390.482 g/kg.

Grand Forks Human Nutrition Research Center. The procedures followed the guidelines of the National Institutes of Health for the experimental use of laboratory animals (*11*).

Production of High-Selenium Soybeans. A soybean cultivar, U03-120221, developed specifically for food-grade markets for the production of tofu and soy milk was used in this study. Soybeans for food uses such as tofu require a higher protein content to increase tofu yield. The seeds from this cultivar meet specifications for tofu production with 37.4% protein on a 13% moisture basis, which is greater than the average of 35.3% for soybeans produced in the United States (*12*). This cultivar was grown at the Agronomy Research Farm, University of Nebraska-Lincoln, during the 2007 crop year. Seeds were planted at 27 viable seeds per meter in a block 12 rows wide and 60 m long, with 0.76 m spacing between rows. Sodium selenate was applied during soybean seed development as a foliar spray at stage R4 (*13*), at a rate of 43.0 g Se/10^4 m^2 using a tractor-mounted sprayer and 114 L of water to deliver the Na_2SeO_4 to the treatment area. An adjacent plot of equal size, separated by 6.1 m of untreated soybeans, was planted on the same date and served as the untreated control. After harvest, the plants were threshed in bulk, seeds cleaned, and analyzed for Se. The raw seeds of the foliar treated soybeans contained 2.91 ± 0.02 mg Se/kg (n = 3), compared with 0.37 ± 0.01 mg Se/kg (n = 3) in the untreated control.

Preparation of Protein Isolate and Tofu. *Protein Isolate*. Soybean seeds were finely ground to flour and defatted using hexane at room temperature. After the removal of the hexane by evaporation, the defatted flour was mixed with water (1:10, w/w) at 32 °C for 15 min, and the mixture was centrifuged, and the soluble portion was collected. The insoluble portion was again mixed with water (1:6, w/w) at 32 °C for 15 min and centrifuged. The soluble extracts from both centrifugations were combined, adjusted with HCl to pH 4.5, and centrifuged again to collect the precipitate. The precipitate was washed by mixing with water (1:6, w/w) at 32 °C for 15 min and centrifuged to collect the curd. The curd was dissolved in water, adjusted with NaOH to pH 7.4, pasteurized at 100 °C for 15 min to denature trypsin inhibitors, and lyophilized to produce the protein isolate.

Tofu. Soybean seeds were soaked in distilled water after which they were mixed with additional warm water at 40 °C (1:5, w/w) and ground thoroughly into a smooth soy milk. The milk was cooked at 100 °C for 15 min and filtered through cheesecloth to remove the pulp. The milk was then coagulated using $CaSO_4$, and the coagulate was filtered to remove water, pressed to make tofu, and then lyophilized.

Table 2. Selenium Content of the Experimental Diets[a]

	targeted Se content (μg/kg)	analyzed Se content (μg/kg)
30% Torula yeast basal diet	5	4.05 ± 1.77
selenium supplement		
selenomethionine	10	10.93 ± 1.27
	20	20.52 ± 1.32
	50	57.68 ± 2.02
	70	79.07 ± 1.18
	100	96.08 ± 0.44
	150	140.82 ± 13.15
protein isolate	20	13.73 ± 1.63
	30	25.08 ± 2.51
	40	32.93 ± 4.29
tofu	20	14.26 ± 0.44
	30	23.87 ± 0.54
	40	34.73 ± 1.12

[a] Values are the means ± SD, n = 21 for the basal diet (5 μg Se/kg), n = 6 for the Se-adequate diet (150 μg Se/kg), and n = 3 for each of the rest diets.

The Se contents of the defatted soy flour, lyophilized protein isolate, and tofu were 3.47 ± 0.31, 5.19 ± 0.12, and 3.0 ± 0.03 mg/kg (n = 5), respectively.

Diet Preparation. A 30% Torula yeast-based diet formulated according to the AIN-93G formulation (*14*) was used as the basal diet in this study, in which the yeast was used as the protein source (**Table 1**). Torula yeast is relatively rich in Fe, P, K, Zn, Mn, and Mg (*15*), and a 30% yeast diet provides an adequate amount of these minerals for rodents. Thus, a mineral mix was prepared containing only those minerals needed to meet National Research Council (NRC) recommendations (*16*) and those included in the AIN-93G formulation (**Table 1**). A Se-adequate diet (positive control) was prepared by adding SeMet to the basal diet to bring the total Se level to 150 μg Se/kg according to the AIN-93G formulation (*14*). Protein isolate was added to the basal diet at 1.93, 3.85, and 5.78 g/kg, and tofu at 3.33, 6.66, and 9.99 g/kg to provide 20, 30, or 40 μg Se/kg diet, respectively. Each mixed diet was analyzed for Se before it was provided to animals (**Table 2**).

Experimental Design. One hundred and eight male weanling Sprague−Dawley rats (strain: SAS:VAF) were obtained from Charles River, Wilmington, MA. Ninety-six rats were fed the basal diet to deplete them of Se; 12 were fed the Se-adequate diet (150 μg Se/kg) as positive controls. The Se depletion was determined by measuring GPX activity in whole blood collected via the tail artery, comparing the results from rats fed the basal diet and the Se-adequate diet. Se depletion was completed after 56 days on the basal diet, and Se-depleted rats were randomly assigned into 13 dietary groups consisting of the basal diet (n = 12) or that diet supplemented with 10, 20, 50, 70, 100, or 150 μg Se/kg from SeMet (n = 8) or 20, 30, or 40 μg Se/kg from the protein isolate or tofu (n = 6). Animals were fed the respective diets for a period of 50 days to replenish Se, which was then assessed as described below.

Rats were individually housed in stainless steel cages with wire-mesh bottoms in an atmosphere of 50% relative humidity at 22 °C with a 12-h light/dark cycle. They had free access to food and deionized water. Rats were weighed weekly, and food intake was recorded on days 74−78 (n = 4 from each group) to determine whether the protein isolate or tofu affected food consumption. At the end of the experiment, all rats were anesthetized with a mixture of ketamine and xylazine. Blood, liver, right gastrocnemius muscle, and kidneys were collected immediately and held at −80 °C for enzyme and Se analyses. Liver, muscle, and kidneys were lyophilized prior to Se analysis.

Enzyme Assays and Selenium Analysis. Glutathione peroxidase activity was determined in whole blood and liver by the method of Paglia and Valentine (*17*) as modified by Lawrence and Burk (*18*) using H_2O_2 as the substrate in the presence of azide. The activity in whole blood was expressed as units/mg hemoglobin (Hb) and in liver as units/mg protein; one unit of activity was defined as the amount of enzyme required to oxidize 1.0 μmol NADPH/min. Thioredoxin reductase activity was determined in the liver by the method of Hill et al. (*19*) as modified by Hintze et al. (*20*). A unit of activity was defined as 1.0 μmol

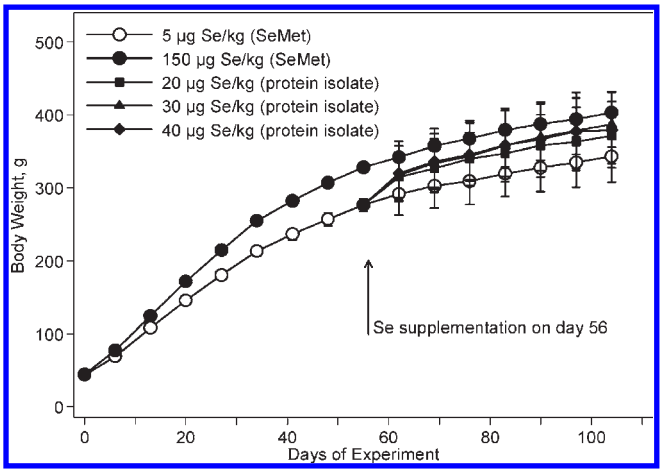

Figure 1. Body weight changes of rats during Se depletion and Se-repletion periods. Student's *t*-test was used to compare the differences between rats fed the basal and the Se-adequate diets throughout the experiment. One-way analysis of variance followed by Tukey contrasts was used to compare differences among the groups during the Se-repletion period. At day 21, the average body weight of rats fed the basal diet (145.8 ± 9.2 g) was significantly less than that of rats fed the Se-adequate diet (171.8 ± 9.2 g); this difference remained statistically significant throughout the experiment. Dietary supplementation with the protein isolate or tofu (data not shown) during the Se-repletion period tended to increase body weight; however, neither increase was significant. Mean ± SD, $n = 12$ for groups fed the basal and the Se-adequate diets and $n = 6$ for groups fed the protein isolate- or tofu-supplemented diets.

thionitrobenzoate formed/(min·mg·protein). Protein was determined by the Bradford method (BioRad). Selenium in diet, plasma, liver, muscle, and kidneys was determined by automated hydride generation−atomic absorption spectrophotometry after nitric-perchloric digestion (*21*); results were expressed as μg/kg for the diet, μmol/L for plasma, and μmol/kg (dry weight) for the liver, muscle, and kidneys.

Statistical Analyses. Student's *t*-test was used to compare differences between groups fed the basal and the Se-adequate diets. One-way analysis of variance followed by Tukey contrasts was used to test differences between the Se-deficient group and the groups fed protein isolate or tofu with different amounts of Se. A slope-ratio model (*22*) was used to determine the relative bioavailability of Se from the protein isolate and tofu compared to Se from SeMet. In this model, linear regression lines are generated for both test and standard compounds, and the relative bioavailability is expressed as the ratio of the slope of the test compound to that of the standard. This model requires that (a) the graded levels of the test compounds are tested; (b) the intake of the test compound does not exceed the amount required to replenish fully the response measure; and that (c) the regression lines for test and reference treatments intercept at a common origin. For all regressions, only the standard response levels between 5 μg Se/kg and 70 μg Se/kg were used because at levels greater than 70 μg Se/kg, the responses for some enzymes and tissue Se had become nonlinear or had reached a plateau. Linearity of the respective regression lines was ascertained for each source of Se after which a single multiple regression model was derived to determine the slope and intercept of the responses for the three Se sources: protein isolate, tofu, and SeMet (*23*). Confidence limits for relative bioavailability were obtained by Fieller's method (*22*). All data are presented as the means ± SD. Differences with $P \leq 0.05$ are considered significant. All statistical analyses were performed using SAS version 9.2 (SAS Institute, Inc., Cary, NC).

RESULTS

The average Se content of the basal diet was 4.05 μg/kg (**Table 2**), which was very low compared with the recommended Se content of the AIN-93G standard diet (150 μg/kg) (*14*). Feeding rats this basal diet retarded growth, which became apparent after the first week of feeding. At day 21, average weight gain of rats fed the basal diet (4.9 ± 0.2 g/d, $n = 12$) was significantly less than that of rats fed the Se-adequate diet (6.1 ± 0.1 g/d, $n = 12$; $P = 0.001$); this difference remained statistically significant throughout the experiment (**Figure 1**). Dietary supplementation with the protein isolate or tofu during the Se-repletion period tended to increase body weight; however, neither increase was significant (**Figure 1**). The average 5-day food intake of all dietary groups was 17.9 g/days after 21 days of Se-repletion, and there was no significant difference in food intake among the groups.

Table 3. Glutathione Peroxidase and Thioredoxin Reductase Activities in Rats Fed the Basal Diet or the Basal Diet Supplemented with the Soy Protein Isolate or Tofu[a]

diet	blood GPX (U/mg Hb)	liver GPX (U/mg protein)	liver TRR (U/mg protein)
	Protein Isolate		
5 μg/kg	11.8 ± 1.6 a	16.0 ± 3.1 a	2.3 ± 0.3 a
20 μg/kg	18.1 ± 1.8 ab	22.4 ± 6.6 a	2.6 ± 0.2 ab
30 μg/kg	26.1 ± 2.1 b	70.1 ± 26.4 b	3.0 ± 0.2 b
40 μg/kg	58.3 ± 12.0 c	137.3 ± 36.9 c	3.6 ± 0.4 c
	Tofu		
5 μg/kg	14.9 ± 2.2 a	16.7 ± 10.3 a	2.1 ± 0.3 a
20 μg/kg	17.7 ± 3.9 a	21.0 ± 6.7 a	2.3 ± 0.3 a
30 μg/kg	24.6 ± 6.7 a	57.5 ± 12.1 b	3.0 ± 0.4 bc
40 μg/kg	63.5 ± 10.3 b	99.0 ± 34.6 c	3.4 ± 0.3 c

[a] One-way analysis of variance followed by Tukey contrasts was used to compare the differences between the Se-deficient group and the groups fed the protein isolate or tofu with different amounts of Se. Values are the means ± SD, $n = 8$ for the basal diet (5 μg Se/kg) and $n = 6$ for each of the rest diets. Values with different letter are statistically significant at $P \leq 0.05$.

Feeding rats the basal diet resulted in Se deficiency. At the 56 day of experimental feeding, blood GPX activity was 34.9 ± 2.8 U/mg Hb ($n = 5$) in rats fed the basal diet and 577.1 ± 27.5 U/mg Hb ($n = 5$; $P < 0.001$) in those fed the Se-adequate diet. At the end of the experiment, blood GPX activity was 11.8 ± 1.6 U/mg Hb ($n = 8$) in rats fed the basal diet, which was 2% of the activity in rats fed the Se-adequate diet throughout the experiment (657.1 ± 149.1 U/mg Hb, $n = 8$). The GPX activity of rats replenished with Se as SeMet at 150 μg Se/kg was 490.5 ± 66.2 U/mg/Hb ($n = 8$). This response represented a 75% restoration in GPX activity compared to the level demonstrated by rats maintained on the Se-adequate diet throughout the experiment. The GPX activity was 196.4 ± 36.0 U/mg Hb ($n = 8$) in the group with SeMet at 50 μg Se/kg; this was 40% of the maximal activity in those with the 150 μg Se/kg repletion. Adding the protein isolate or tofu to the basal diet resulted in a dose-dependent increase in GPX activity (**Table 3**). Those responses were 96.5% and 98.3%, respectively, of that observed for SeMet (**Table 4**).

Hepatic GPX activity responded to changes in dietary Se in a manner similar to that observed for blood GPX activity (**Table 3**). At the end of the experiment, hepatic GPX activity was 16.0 ± 3.1 U/mg protein in rats fed the basal diet which represented < 1% of the activity in rats fed the Se-adequate diet throughout the experiment (3493.6 ± 359.0 U/mg protein, $n = 8$). The enzyme activity in rats replenished with SeMet at 150 μg Se/kg (3546.7 ± 762.3 U/mg protein, $n = 8$) was similar to that of rats maintained on the same diet throughout the experiment, representing 100% restoration. Dietary supplementation with the protein isolate or tofu resulted in a dose-dependent increase in liver GPX activity (**Table 3**), indicating bioavailabilities of 102.8% (protein isolate) and 85.7% (tofu) relative to SeMet (**Table 4**).

Hepatic TRR activity responded to changes in dietary Se (**Table 3**). At the end of the experiment, TRR activity was

Table 4. Relative Biological Value (RBV; Bioavailability) of Selenium from Soy Protein Isolate and Tofu to Selenomethionine in Rats[a,b]

	% RBV (95% confidence interval)	
	protein isolate	tofu
blood GPX	96.5 (88.9, 104.2)	98.3 (88.5, 108.0)
liver GPX	102.8 (94.2, 111.3)	85.7 (73.3, 98.2)
liver TRR	64.2 (46.1, 82.4)	70.2 (46.2, 94.2)
plasma Se	85.6 (77.4, 93.6)	100.6 (92.9, 108.3)
liver Se	113.0 (102.8, 123.2)	110.6 (101.1, 120.2)
muscle Se	120.6 (109.1, 132.1)	119.9 (105.9, 134.0)
kidney Se	99.5 (93.2, 105.8)	96.3 (90.1, 102.4)
mean ± SD[c]	97.5 ± 18.5	97.4 ± 16.2

[a] Standard response curve for Se dependent enzyme activities and tissue Se was made by feeding rats diets containing various contents of Se as SeMet. The enzyme activities and tissue Se of rats fed the protein isolate- or tofu-supplemented diet were compared with the values on the standard response curve. [b] The % RBV was estimated by using the slope-ratio method (*22*) for enzyme activities or the parallel line assay for tissue Se (*22*). [c] The overall mean of each % RBV column.

2.3 ± 0.3 U/mg protein (n = 8) in Se-deficient rats, which was 29% of that in Se-adequate rats (8.0 ± 0.4 U/mg protein, n = 8). Enzyme activity was 7.9 ± 0.2 U/mg protein (n = 8) in rats replenished with SeMet at 150 μg Se/kg, representing 100% restoration in TRR activity. Dietary supplementation with the protein isolate or tofu resulted in a dose-dependent increase in hepatic TRR activity (**Table 3**), indicating bioavailabilities of 64.2% (protein isolate) and 70.2% (tofu) relative to SeMet (**Table 4**).

Feeding rats the basal diet depleted tissue Se. At the end of the experiment, Se was 0.14 ± 0.10 μmol/L in plasma and 0.49 ± 0.16, 0.05 ± 0.07, and 4.24 ± 0.73 μmol/kg (dry weight) in the liver, muscle, and kidneys, respectively, in Se-deficient rats, whereas Se was 6.52 ± 0.63 μmol/L in plasma and 40.01 ± 4.79, 7.47 ± 0.61, and 72.22 ± 5.67 μmol/kg in the liver, muscle, and kidneys, respectively, in Se-adequate rats. Dietary supplementation with SeMet during the Se-repletion period resulted in a dose-dependent increase in tissue Se (**Figure 2**). At the level of 150 μg Se/kg, Se was 6.14 ± 0.87 μmol/L in plasma and 35.62 ± 3.72, 5.82 ± 0.94, and 65.04 ± 7.02 μmol/kg in the liver, muscle, and kidneys, respectively, representing 94.2%, 89.0%, 78.0%, and 90.1% restoration of Se compared with that of the rats maintained on the Se-adequate diet throughout the experiment. At the 50 μg Se/kg diet, Se was 3.34 ± 0.46 μmol/L in plasma and 14.69 ± 2.04, 2.57 ± 0.29, and 42.08 ± 2.79 μmol/kg in the liver, muscle, and kidneys, respectively, which were approximately 54.4%, 41.3%, 44.1%, and 64.7% of those observed in rats with the 150 μg Se/kg repletion. Adding the protein isolate or tofu to the basal diet during the Se-repletion period resulted in dose-dependent increases in plasma, liver, muscle, and kidney Se (**Figure 2**). The relative bioavailabilities of Se from the protein isolate were 85.6% (plasma), 113.0% (liver), 120.6% (muscle), and 99.5% (kidneys), and those of Se from tofu were 100.6% (plasma), 110.6% (liver), 119.9% (muscle), and 96.3% (kidneys), respectively, compared to that from SeMet (**Table 4**).

DISCUSSION

In the present study, we assessed the bioavailability of Se from soy protein isolate and tofu on the basis of the restoration of Se-containing enzyme activities and tissue Se contents in Se-deficient rats, using SeMet as a reference. We produced low-Se status in rats by feeding them a basal diet based on 30% Torula yeast for a sufficient time and then replenished Se by supplementing their diets with the protein isolate or tofu, and we used the slope-ratio model (*22*) to assess the bioavailability of Se from these soy products. We previously demonstrated the usefulness of this method in evaluating the bioavailability of Se from foods

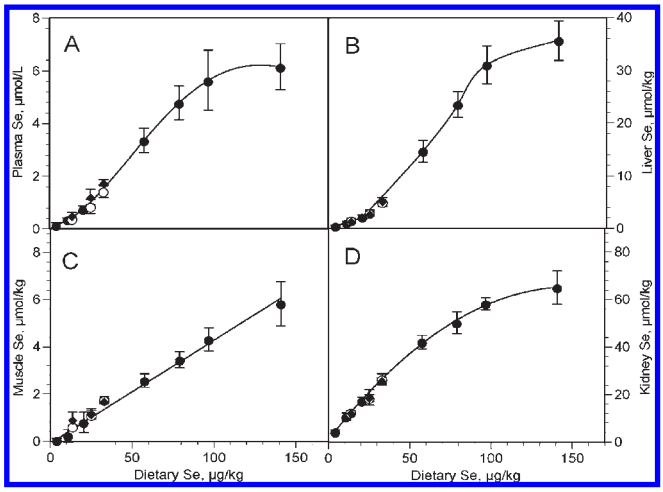

Figure 2. Response curves of plasma (**A**), liver (**B**), muscle (**C**), and kidneys (**D**) to dietary supplementation with SeMet (●), protein isolate (○), and tofu (♦). Mean ± SD, n = 8 for SeMet supplementation and n = 6 for protein isolate or tofu supplementation.

(*21*, *24*). We found in the present study that consumption of protein isolate- and tofu-supplemented diets resulted in dose-dependent increases in restoring Se-containing enzyme activities and tissue Se contents in Se-deficient rats in a manner similar to that of SeMet.

There are two major Se compartments in the body: (a) a selenocysteine (SeCys) compartment comprising a small number of proteins containing Se incorporated as SeCys by a highly specific cotranslational process (*25*−*27*) and (b) a nonspecific SeMet compartment comprising general proteins in which SeMet is incorporated as a mimic of its sulfur analogue methionine. Whereas the SeCys compartment is regulated by the availability of Se at low levels of intake, the SeMet compartment is relatively large, potentially including all sites of methionine in body proteins. The nonspecific incorporation of SeMet is not specifically regulated. The dominant form of Se in plant foods including soy is SeMet (*28*−*30*), which upon its absorption can be nonspecifically incorporated into the SeMet compartment or metabolized via enzymatic or nonenzymatic steps through selenide to enter the SeCys compartment or be methylated and excreted.

We analyzed GPX and TRR activities in the present study as a measurement of Se from the protein isolate and tofu for incorporation into the SeCys compartment for specific seleno-protein synthesis. We found dietary supplementation with either the protein isolate or tofu produced dose-dependent increases in blood and hepatic GPX activities that were similar to those of SeMet, indicating that Se from these three sources is comparably efficient in its digestion, absorption, and metabolic conversion to functional SeCys enzymes. Glutathione peroxidase is the most commonly used indicator of Se status in humans and animals. Our results were consistent with previous reports that Se from Se-enriched foods is bioavailable in restoring GPX activity (*31*, *32*). Our results of hepatic TRR were in agreement with previous studies that this enzyme is less sensitive to changes in dietary Se (*33*). In the present study, the hepatic TRR was less sensitive to Se depletion and Se supplementation than blood and liver GPX, which resulted in a relatively lower bioavailability compared with that of GPX. While both tissue Se and seleno-enzyme activities responded to the amount of bioavailable Se consumed, restoration of SeCys enzyme activities in Se-depleted animals has particular physiological relevance by indicating biological functions.

The Se contents of blood, liver, muscle, and kidneys constitute 60% of total body Se in humans (*34*). In the present study, we found that Se from either the protein isolate or tofu was also comparable to Se from SeMet with respect to its capability of replenishing Se contents of the plasma, liver, muscle, and kidneys. Increases in Se contents of these tissues are most likely due to the nonspecific incorporation of SeMet into proteins, expending the SeMet compartment. That we observed increases in both selenoenzyme activities and tissue Se levels from all three sources of dietary Se indicates that in the Se-depleted animals, SeMet is not completely catabolized to selenide to enter the SeCys compartment but that a significant portion remains in the nonspecific SeMet compartment.

In the present study, Se from each of these sources was comparably effective in restoring Se-containing enzyme activities and tissue Se content. Soy protein isolate is a highly refined protein product with most nonprotein components removed, whereas tofu coagulated from soy milk is a crude protein product retaining lipids and carbohydrates. A major difference between these two products is the protein content. Our protein isolate contained more Se than the defatted soy flour, which was higher in Se than the raw seeds. However, Se content of tofu was very similar to that of the raw seeds. These findings are consistent with a previous report that Se content of processed soy products is dependent on the protein levels of such products (*35*). Thus, different methods in processing soy foods may affect protein and Se contents but not the bioavailability of Se.

We found in the present study that there was significant growth retardation in rats fed the basal diet. These results were different from our previous studies from which we found no significant difference in body weight between rats fed the basal and the Se-adequate diets (*21*, *24*). We chose Torula yeast as the protein source for those diets because it was very low in Se. We balanced the diet with all of the required nutrients including sulfur-containing amino acids, minerals, and vitamins to meet the NRC recommendations (*16*) and the AIN-93G formulation (*14*). Thus, the only difference between the basal diet and experimental diets was their amounts and sources of Se. We noted a nonsignificant tendency for increased growth in rats fed the protein isolate- or tofu-supplemented diets during the Se-repletion period. This indicates that the quantity of Se in the diet is responsible for the differences in growth rate among the groups in this experiment.

Both the research community and general public have great interest in soy and Se for their nutritional values and potential health benefits. Soy consumption is associated with a reduction in cancer risk (*7*) and an improvement in blood lipid profiles which benefits cardiovascular health (*6*). Diabetes has been an area of interest for both soy and Se research. The National Health and Nutrition Examination Survey (*36*) shows that high serum Se ($\geq$137.66 ng/mL) is associated with the prevalence of diabetes in a sample of the U.S. population; however, the Selenium and Vitamin E Cancer Prevention Trial (*37*) shows that Se supplementation is not associated with a significant increase in the risk of type 2 diabetes mellitus (relative risk, 1.07; 99% CI, 0.94−1.22, $P = 0.16$). A small scale clinical study (*38*) demonstrates that Se reduces the development of vascular complications in type 2 diabetic patients by reducing nuclear factor-κ B activity, and laboratory studies show that Se treatment alleviates late diabetic complications in a diabetic rat model (*39*). Epidemiologic studies demonstrate that soy intake is inversely associated with the risk of type 2 diabetes mellitus in humans (*40*), and clinical studies show that soy protein improves renal functions in diabetic patients (*41*, *42*). Selenium is one of the most extensively studied nutrients for its potential health benefits. It has been documented that Se improves immune defense in humans (*43*) and animals (*44*). The present study demonstrates that Se from high-Se soybeans is highly bioavailable in the rodent model we studied. It paves the road for further investigations of nutritional values and health benefits of high-Se soybeans in an additive and/or synergetic manner, including its roles in diabetes treatment and/or prevention.

In conclusion, our study demonstrates that Se is highly bioavailable from the protein isolate and tofu prepared from the high-Se soybean cultivar we developed and that different processing methods do not affect the Se bioavailability of soy foods. We would expect similar results from naturally produced high-Se soybeans. Because Se deficiency remains a health concern in East Asia (*45*) and Europe (*46*) where Se is not generally available from food sources, soybeans produced from Se-rich soils of the Northern Plains may be a good dietary source of Se, particularly for those countries.

ABBREVIATIONS USED

Se, selenium; SeMet, selenomethionine; GPX, glutathione peroxidase; TRR, thioredoxin reductase; SeCys, selenocysteine.

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