JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Determination of Selenium Bioavailability from Wheat Mill Fractions in Rats by Using the Slope-Ratio Assay and a Modified Torula Yeast-Based Diet

PHILIP G. REEVES,*,† BRIAN R. GREGOIRE,† DAVID F. GARVIN,§ GARY A. HARELAND,# JAMES E. LINDLAUF,† LUANN K. JOHNSON,† AND JOHN W. FINLEY†,‡

Grand Forks Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Grand Forks, North Dakota 58203; Plant Science Research Unit, Agricultural Research Service, U.S. Department of Agriculture, and Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minnesota 55108; and Hard Red Spring and Durum Wheat Quality Laboratory, Red River Valley Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, Fargo, North Dakota 58105

Selenium is an essential mineral micronutrient for animals, and significant evidence supports an association between supranutritional Se intake and a reduction in the incidence of some forms of cancer. Thus, supplemental Se intake may provide an avenue for reducing cancer incidence. However, an important issue to consider is the form of Se that should be provided in such a supplement, because the bioavailability and bioactivity of Se can vary dramatically depending on the chemical form in which it is delivered. Because wheat products are the largest source of Se in U.S. diets, the absorption of Se was evaluated in different fractions of milled wheat that exhibits very high Se levels, owing to its production on naturally Se-rich soils. An experiment was conducted to determine the bioavailability of Se from three milled fractions of high-Se wheat. The method used was the slope-ratio assay, which measures the ability of Se from the wheat fractions to regenerate Se-dependent enzyme activities and tissue Se concentrations in Se-deficient rats. The responses generated from wheat Se were compared to a standard response curve generated by feeding graded amounts of Se as sodium selenite (Na_2SeO_3; NaSelenite) or selenomethionine (SeMet) in an AIN-93G-Torula yeast-based diet. Results showed that Se from wheat flour (~75% extraction) was nearly 100% available by a number of measures including plasma, liver, kidney, and muscle Se concentrations and liver and erythrocyte Se-dependent enzyme activities when compared with similar measures in rats fed NaSelenite or SeMet. However, on the basis of similar criteria, Se from wheat shorts was only about 85% available and that from wheat bran was about 60% available for absorption. These results indicate that high-Se wheat products, mainly those made from refined flour alone, might be particularly well suited for use as dietary Se supplements.

KEYWORDS: Rats; selenium; selenium bioavailability; Torula yeast; wheat bran; wheat flour; wheat shorts

INTRODUCTION

Selenium is an essential nutrient in both humans and animals, and a dietary deficiency of the mineral results in Keshan disease in humans and white muscle disease in domestic livestock (*1*–*3*). Se plays an essential role at the catalytic site of multiple selenoproteins, including glutathione peroxidase, thioredoxin reductase, and the iodothyronine selenodeiodinases (*2*). Se intakes of ~200 μg/day have been shown to reduce the risk of cancer, especially prostate (*4*) and colon (*5*) cancers. These findings have led to proposals to increase the Se intake of populations at risk. Numerous Se supplements are currently available in the marketplace; however, the American Dietetic Association recommends that nutrients be consumed through foods whenever possible (*6*), but the availability of food nutrients for absorption and assimilation can be a limiting factor.

Assessing the bioavailability of Se can be complex because there are multiple naturally occurring chemical forms of this

* Address correspondence to this author at the Grand Forks Human Nutrition Research Center, USDA-ARS, 2420 Second Ave. N., Grand Forks, ND 58203 [telephone (701) 795-8497; fax (701) 795-8395; e-mail preeves@gfhnrc.ars.usda.gov].

† Grand Forks Human Nutrition Research Center, U.S. Department of Agriculture.

§ U.S. Department of Agriculture and University of Minnesota.

Hard Red Spring and Durum Wheat Quality Laboratory, U.S. Department of Agriculture.

‡ Present address: A. M. Todd, Inc., Montgomeryville, PA 18936.

10.1021/jf062572u CCC: $37.00 © 2007 American Chemical Society
Published on Web 12/19/2006

Table 1. Composition of the Modified Basal AIN-93G Torula Yeast Diet

ingredient	g/kg
cornstarch[a]	427.41
torula yeast[b]	300.00
sucrose[c]	100.00
soybean oil[d]	70.00
cellulose[b]	50.00
low Se mineral mix (see **Table 2**)	35.00
AIN-93G vitamin mix[b]	10.00
L-cystine[e]	3.52
L-methionine[e]	2.91
choline bitartrate[b]	1.00
L-tryptophan[e]	0.16

[a] Roquette America, Keokuk, IA. To obtain the required amount of Se in the diet (20, 30, or 40 μg/kg) from the wheat fraction the following amounts of each fraction were added at the expense of cornstarch: flour, 2.92, 4.38, 5.84 g/kg; shorts, 1.91, 2.87, 3.83 g/kg; bran, 1.65, 2.47, 3.29 g/kg. [b] Harlan Teklad, Madison, WI (*12*). [c] United Sugars, Minneapolis, MN. [d] ConAgra Foods, Irvine, CA. [e] Ajinomoto, Raleigh, NC.

Table 2. Composition of the Mineral Mix for the Modified Torula Yeast Diet[a]

mineral	g/kg
calcium carbonate, anhydrous, 40.04% Ca	555.260
sodium chloride, 39.34% Na	52.17
sodium metasilicate, 9 hydrate, 9.88% Si	1.450
chromium potassium sulfate, 12 hydrate, 10.42% Cr	0.275
copper carbonate, 57.47% Cu	0.143
boric acid, 17.5% B	0.082
sodium fluoride, 45.24% F	0.064
nickel carbonate, 45% Ni	0.032
lithium chloride, 16.38% Li	0.017
potassium iodate, 59.3% I	0.010
ammonium paramolybdate, 4 hydrate, 54.34% Mo	0.008
ammonium vanadate, 43.55% V	0.007
powdered sucrose	390.483

[a] Because of the inherently high concentrations of P, Mg, K, Fe, Zn, and Mn in the Torula yeast, none of these minerals was added to the mineral mix.

element in nature. These include Se salts, Se derivatives of sulfur amino acids, and methylated derivatives of selenoamino acids. The chemical form of Se partially determines its metabolism and its ultimate biological action; therefore, unlike most other nutrients, the bioavailability of Se cannot be estimated exclusively by measuring absorption. Instead, bioavailability must be assessed by the ability of a particular Se compound to be transformed into a metabolically active form of Se (*7*). Functional bioassays, such as activity restoration of the selenoprotein glutathione peroxidase (GPx1) in Se-deficient laboratory animals, are among the most commonly used methods to assess the bioavailability of Se from food (*7*). However, restoration of organ Se concentrations and activity of thioredoxin reductase also have been used to assess Se bioavailability (*8*).

Wheat products are the largest single source of Se in U.S. diets (*9*). When grown on certain seleniferous soils found in some regions of the United States, particularly North Dakota and South Dakota, wheat can accumulate very high concentrations of Se in the grain (*10*). If available for absorption, breads and other products made from this grain could provide an excellent highly concentrated source of dietary Se for human and animal consumption. The following experiment was designed to determine the bioavailability of Se from different mill fractions of such high-Se wheat.

MATERIALS AND METHODS

This study was approved by the Animal Use Committee of the USDA-ARS, 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*).

Diet Preparation. The base formula of the AIN-93G diet (*12*) and the average mineral and amino acid contents of Torula yeast were used to derive the basal diet composition, which is shown in **Table 1**. In order for the yeast to supply enough essential amino acids without excessive supplementation, it was fed at 30% of the diet. However, at this concentration, there were insufficient amounts of the sulfur amino acids and tryptophan to equal that recommended for rodents by the NRC (*13*); thus, a supplement containing L-methionine, L-cystine, and L-tryptophan was added to the diet. At 30% of the diet, the yeast will provide an excess of Fe, P, K, Zn, Mn, and Mg, and no additions of these minerals to the diet are required. Thus, a premix was prepared with only those minerals naturally contained in the ingredients of this diet that did not meet the NRC requirement for the rat (**Table 2**).

Addition of Wheat Fractions to the Diet. High-Se hard red winter wheat, variety Nekota, was obtained from a farm near Milesville, SD, and fractionated according to the following procedure: Forty pounds of cleaned grain were tempered to 16.0% moisture basis, conditioned for approximately 16 h, and milled in a Bühler laboratory experimental mill (MLU-202) according to method 26-21A of the AACC International (*14*). The tempered wheat was metered at a feed rate of approximately 175 g/min and milled to obtain approximately 75% extraction of straight grade flour. In the milling operation, three break flour streams (B1, B2, B3), three reduction flour streams (R1, R2, R3), a shorts fraction, and a bran fraction were obtained. The patent flour (PF) used in this study was prepared from a blend of the three break flour and the three reduction flour streams in proportions according to the weight percentages of each flour stream that was obtained in the milling operation.

Each wheat fraction to be tested was mixed with water and cooked before it was incorporated into the diet. Briefly, 200 g of each fraction was added to 900 mL of deionized water in a Pyrex container and boiled at low heat on a stovetop for 15 min. Each of the cooked porridges was lyophilized and ground to a powder in a centrifugal grinder. Each powder was thoroughly mixed and stored at 4 °C until incorporated into the diet. By analysis, the cooked, lyophilized material contained Se in the following concentrations (mean ± SD, $n = 4$): flour, 6.9 ± 0.3 mg/kg; shorts, 12.1 ± 0.1 mg/kg; bran, 10.4 ± 0.1 mg/kg. The Se is incorporated primarily into the protein component in the form of SeMet (*15*, *16*). Indeed, these values reflect the protein content of each fraction of 10.9, 16.5, and 15.8%, respectively.

Experimental Design. One hundred and eighty-six male weanling Sprague−Dawley rats [strain SAS:VAF(SD); Sasco, Ballard, MA] were fed the basal Se-deficient diet for 41 days. Then five of the rats were randomly selected and killed to determine whether they were Se-deficient by measuring liver and erythrocyte GPx1 activity and plasma Se concentration. The results were compared with those from five rats that had received diets containing 150 μg of Se/kg for the same period. After the results showed that the rats were definitely Se-deficient, the remaining rats were divided into 22 groups of 8 rats each. Six groups were fed a similar diet as before, but supplemented with sodium selenite (Na_2SeO_3; NaSelenite), and six groups were fed selenomethionine (SeMet). The target concentrations of added Se ranged from 10 to 150 μg/kg of diet (**Table 3**). Another group of eight rats continued to receive the Se-deficient diet, and another group continued receiving an adequate Se diet throughout the study.

To test the bioavailability of Se from the various wheat fractions, three groups of eight Se-deficient rats were fed a diet containing cooked wheat flour at three different levels to obtain three concentrations of Se. Three more groups of Se-deficient rats were fed diets containing cooked wheat shorts to obtain three levels of dietary Se, and three additional groups were fed diets with wheat bran. These fractions were added at the expense of small portions of starch. The results of the enzyme activities and tissue Se concentrations of rats consuming these diets were compared to values on the standard response curve by using the analyzed diet values as the independent variable. The slope-ratio

Table 3. Concentration of Se in Experimental Diets

Se supplement	target Se concn (μg/kg)	Se concn by analysis[a] (μg/kg)
sodium selenite	5[b]	2.9 ± 0.5
	10	11.4 ± 3.0
	20	20.3 ± 5.1
	50	47.1 ± 2.7
	70	63.4 ± 2.6
	100	86.6 ± 15.3
	150	141.8 ±12.6
selenomethionine	5[b]	2.9 ± 0.5
	10	15.3 ± 1.5
	20	19.2 ± 6.4
	50	43.4 ± 3.3
	70	58.1 ± 7.4
	100	93.9 ± 8.14
	150	137.1 ± 18.4
flour	20	23.4 ± 0.8
	30	33.0 ± 6.1
	40	44.4 ± 1.9
shorts	20	19.8 ± 0.6
	30	30.4 ± 2.2
	40	39.8 ± 3.6
bran	20	23.5 ± 1.1
	30	29.5 ± 2.5
	40	46.8 ± 3.8

[a] Mean ± SD of four replicates. [b] These two treatments are the same, because no sodium selenite or selenomethionine was added to the diets.

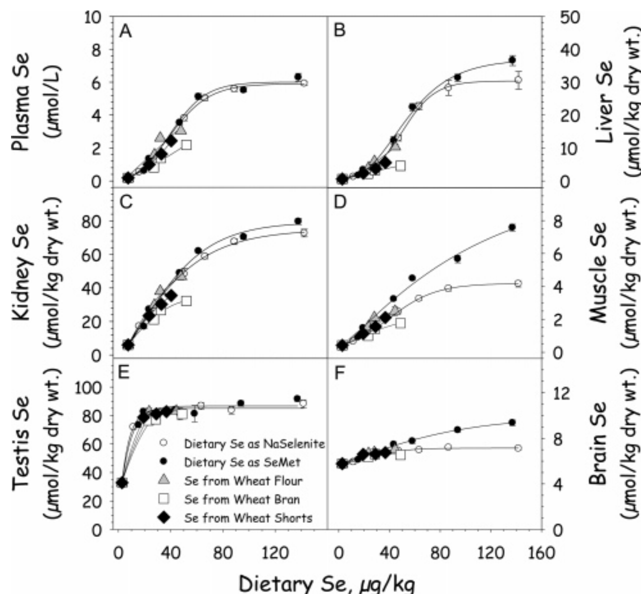

Figure 1. Response curves of plasma and organ concentrations of Se-deficient rats fed Se as increasing concentrations of sodium selenite (NaSelenite) or selenomethionine (SeMet) in the diet. Wheat flour, shorts, and bran were fed to Se-deficient rats at three concentrations each and the results compared with values in the response curves. Points represent the mean ± SEM for eight replicates. In some cases, SEM bars are hidden within the symbols.

assay was used to express the bioavailability of Se from these products relative to NaSelenite or SeMet.

The rats were individually housed in hanging stainless steel cages with wire-mesh bottoms in an atmosphere of 50% relative humidity at 22 °C and with a 12-h light−dark cycle with light beginning at 6:00 a.m. Food was offered ad libitum in glass containers with stainless steel screw caps with holes to allow access to the food, but to limit food wastage. Deionized water was offered ad libitum in glass bottles with silicon stoppers and stainless steel sipper tubes. Food and water were monitored daily for freshness, and rats were weighed weekly throughout the experiment. After 50 days of Se supplementation, all rats were anesthetized with a mixture of ketamine (100 mg/mL) and xylazine (20 mg/mL) in a ratio of 1.37:1. One milliliter of the mixture per kilogram of body weight was injected intraperitoneally. Blood (∼10 mL) was withdrawn from the abdominal aorta into EDTA-coated tubes until the rat expired. Blood samples were centrifuged to obtain plasma for Se analysis, and the red cells (RBC) were used for GPx1 activity determinations. Livers, kidneys, testes, sartorius muscles, and brains were collected and frozen in liquid nitrogen and stored at −80 °C for later determination of Se concentration. Livers also were assayed for GPx1 and TRR activities.

Enzyme and Se Assays. Glutathione peroxidase activities in RBC and liver were determined according to the method of Paglia and Valentine (*17*). Thioredoxin reductase (TRR) activity in liver was determined spectrophotometrically by using the method of Hill et al. (*18*) as modified by Hintze et al. (*19*). Selenium was analyzed in various organs by procedures outlined by Reeves et al. (*8*). Standard reference materials (National Institute of Standards and Technology, Gaithersburg, MD) consisting of durum wheat flour (NIST-SRM 8436) and bovine liver (NIST-SRM 1577b) were included in each batch of samples for wheat fractions and various animal tissues, respectively, to ensure accurate analyses of Se.

Statistical Analyses. The relative bioavailability of Se in wheat bran, flour, and shorts was determined relative to either NaSelenite or SeMet by using the slope-ratio method (*20*, *21*). The tissue and plasma Se data were linearly related to the intakes of NaSelenite or SeMet; the relationships between the enzyme data and intakes of NaSelenite or SeMet were linearized by using log-linear regression models. For all regressions, only the standard response doses between 3 and 75 μg of Se/kg of diet were used, because at doses >75 μg of Se/kg of diet, the responses had reached a plateau and were not affected further by increasing Se intakes. For each set of criteria evaluated, the linearity of the regression lines was ascertained for each source of Se separately. Then, a single multiple-regression model was derived to determine the slope and intercept of the responses for wheat bran, flour, or shorts and either NaSelenite or SeMet with the "no added Se" group serving as the control (*21*). Ninety-five percent confidence intervals (CI) for relative bioavailability were obtained by using Fieller's method (*20*). Data are reported as means ± standard deviation (SD) in **Table 3**, as means ± standard error of the mean (SEM) in **Figures 1** and **3**, and as means with 95% confidence intervals (CI) in **Figure 2**. A value of $P \le 0.05$ was considered to be statistically significant. For data in **Figure 2**, if the 95% CIs do not overlap, the differences between RBV estimates are statistically significant ($P \le 0.05$). The estimated overall RBV was determined by taking the overall average of all bioavailable parameters. All statistical analyses were done by using SAS/Stat version 9.1 (SAS Institute, Inc., Cary, NC).

RESULTS

The basal diet was very low in Se, containing 2.9 ± 0.5 μg/kg. The rate of weight gain for each group of rats was the same throughout the study (data not shown). They averaged 7 g of gain/day for the first 4 weeks of the study. The overall average gain for the 13 weeks was 3.5 g/day with no significant differences among groups. To determine if the different wheat fractions affected food consumptions, food intake measurements were made on days 52−55. Compared with the group consuming a diet with 150 μg of Se/kg as NaSelenite throughout the study, there were no effects of wheat fractions on food intake. All groups averaged from 6.1 ± 1.0 g to 7.1 ± 1.0 g per 100 g of body weight per day.

Signs of Se deficiency, as indicated by plasma Se concentration and liver GPx1 activity, were apparent after 41 days of feeding the Se-deficient diet. Plasma Se concentrations were 3% and liver GPx1 activity only 1.5% of the control values. Supplementing the diet with as little as 11 μg of Se/kg caused plasma Se to rise off baseline and to maximize near 100 μg of

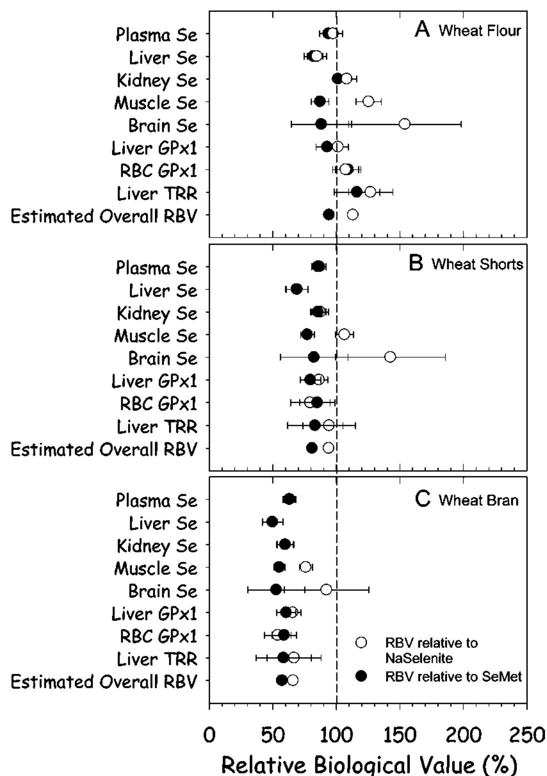

Figure 2. Biological value (RBV; bioavailability) of Se from wheat flour, wheat shorts, and wheat bran in rats relative to Se from sodium selenite (NaSelenite) or selenomethionine (SeMet). Standard response curves for tissue Se and Se-dependent enzyme activities were made by feeding various dietary concentrations of Se as NaSelenite or SeMet to Se-deficient rats. Tissue Se and enzyme activities from Se-deficient rats fed wheat fractions with known concentrations of Se were compared with the values on the standard response curves. Percentage RBV was estimated by using the slope-ratio assay method. Points represent the means with 95% confidence intervals (CI) of eight replicates. CIs that do not overlap are significantly different from each other ($P < 0.05$).

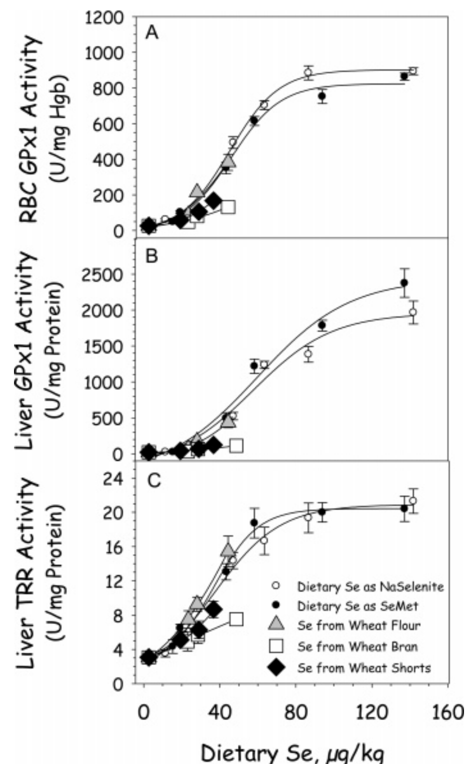

Figure 3. Response curves of blood and liver GPx1 activity and liver TRR activity in Se-deficient rats fed Se as increasing concentrations of sodium selenite (NaSelenite) or selenomethionine (SeMet) in the diet. Wheat flour, shorts, and bran were fed to Se-deficient rats at three concentrations each and the results compared with values on the response curves. Points represent the mean ± SEM for eight replicates.

Se/kg of diet (**Figure 1A**). By using plasma Se, the bioavailability of Se from wheat flour relative to both NaSelenite and SelMet was >90% (**Figure 2A**). The bioavailability of Se from wheat shorts relative to the standards was >85%, which was not significantly ($P > 0.05$) different from wheat flour. However, bioavailability from wheat bran was only 60% (**Figure 2C**) whether compared with NaSelenite or SeMet. This value was significantly ($P < 0.05$) different from both wheat flour and shorts.

The liver Se concentration curves were sigmoidal in shape and, although not determined mathematically, they seemed to reach 50% of maximal at about 50 μg of Se/kg of diet (**Figure 1B**). However, the curves were still rising at the maximal dietary concentrations of Se when SeMet was used as the supplement. The bioavailability of Se from wheat flour was >80% of that from NaSelenite or SeMet (**Figure 2A**). However, Se from wheat shorts was only 70%, but not different ($P > 0.05$) from flour (**Figure 3B**). Se from wheat bran was only 50% as available as that in NaSelenite or SeMet, which was significantly ($P < 0.05$) different from both flour and shorts (**Figure 2C**). On the basis of kidney Se, the relative bioavailability of Se from wheat flour was 100% of that in both NaSelenite and SeMet (**Figures 1C** and **2A**). The relative bioavailability from wheat shorts was >85%, but only 60% available from wheat bran (**Figure 2B**,**C**, respectively). Again, the availability of Se from bran was significantly ($P < 0.05$) lower than that from flour or shorts.

Dietary Se as SeMet increased muscle Se concentration to nearly twice that of rats fed Se as NaSelenite (**Figure 1D**). However, in rats that consumed a diet with 150 μg of Se/kg as NaSelenite throughout the study period, muscle Se was not different from that of the SeMet group (data not shown). Relative bioavailability of Se from wheat flour was 125% of that from NaSelenite, but only 87% of that from SeMet ($P < 0.05$; **Figure 2A**). Bioavailability from wheat shorts was 100% relative to NaSelenite and 77% relative to SeMet ($P < 0.05$). From wheat bran, it was 76 and 55%, respectively ($P < 0.05$). Bioavailability from wheat bran was less ($P < 0.05$) than that from flour or shorts.

Se concentration in testis seems to maximize at around 11 μg of Se/kg of diet (**Figure 1E**), and no relative bioavailability values could be calculated. Se in brain was not severely depleted by feeding Se-deficient diets, and increased only slightly upon refeeding Se (**Figure 1F**). The rate of increase in brain Se seemed to be greater in rats fed SeMet than in those fed NaSelenite. Bioavailability of Se from wheat flour and shorts relative to NaSelenite was largely overestimated, with values of 154 and 142%, respectively (**Figure 2A**,**B**). Relative to SeMet, bioavailability was 88 and 82%, respectively, but because of the high variability, there were no significant differences.

The activity of GPx1 in RBC and liver was not different from baseline until dietary Se had reached values between 20 and 50 μg/kg (**Figure 3A**,**B**, respectively). At approximately 50 μg of Se/kg of diet, RBC Gpx1 activity had reached about 50% of

the maximal activity. The titration curve for liver GPx1 activity was similar to that for RBC GPx1, except that 50% of maximal activity was not reached until dietary Se was at least 60 μg/kg (**Figure 2B**). However, activity seemed to be maximized at 140 μg/kg. Bioavailability of Se from wheat flour as estimated by the change in activities of these enzymes was about 95−110% relative to either NaSelenite or SeMet, but not different between the Se sources ($P > 0.05$; **Figure 2A**). Bioavailability from wheat shorts ranged from 80 to 86% (**Figure 2B**), and from wheat bran, it ranged from 40 to 60% (**Figure 2C**). Bioavailability from wheat shorts was significantly ($P < 0.05$) different from wheat bran.

The activity of thioredoxin reductase (TRR), another Se-dependent enzyme in liver, also was affected by dietary Se concentrations (**Figure 3C**). Both NaSelenite and SeMet added to the diet of Se-deficient rats increased activity, which seemed to reach 50% of maximal at approximately 30 μg of Se/kg of diet. Maximal activity seemed to be reached at approximately 90 μg of Se/kg of diet. Relative bioavailability of Se from wheat flour by using this measure was estimated at over 100%, compared with either NaSelenite or SeMet, but there was no difference between the two ($P > 0.05$; **Figure 2A**). Bioavailability from wheat shorts ranged from 83 to 94% and was not different from flour ($P > 0.05$; **Figure 2B**). From wheat bran, bioavailability ranges only from 58 to 67% ($P > 0.05$; **Figure 2C**). This was significantly ($P < 0.05$) different from wheat flour but not from wheat shorts.

DISCUSSION

The bioavailability of Se in various wheat fractions was estimated by comparing Se from these fractions with that supplied as NaSelenite and as SeMet in their potential to restore tissue Se concentrations and selenoenzyme activities in Se-deficient rats. The slope-rate assay method was used to make the final calculations (*20*). This assay has been used in the past to determine the bioavailability of Se from numerous foods and food sources including buckwheat bran (*8*), wheat (*22*), beef (*23*), Se-enriched yeast (*24*), Se-enriched algae (*25*), Se-enriched broccoli (*26*), and salmon (*27*). Two basic assumptions in this assay are that the diet contains graded amounts of Se and that the intake of Se does not exceed the amount required to fully replete the response parameters (*27*). This was accomplished by feeding wheat fractions that contained a higher than normal concentration of Se, because of its production on high-Se soils. No wheat fraction of >0.6% of the diet was required to supply the desired amount of Se and to make the test response fit on the up-slope of the linear portion of the standard response curve. Therefore, it was not necessary to incorporate large quantities of each wheat fraction into the diet, which could possibly alter digestibility and affect the bioavailability of Se (*28*).

The wheat milling process entails the physical separation of different parts of the wheat grain to produce flour. Additional non-flour mill fractions are recovered during milling as well; these are variously composed of the other cell layers and structures present in the intact wheat grain. The different mill fractions thus have distinct chemical compositions. The endosperm constitutes approximately 80% of the mass of the intact grain and is converted into flour during milling. Flour consists primarily of starch, but also contains a significant amount of protein, and has little fiber. The milling process disrupts the cell structure of the endosperm. The bran fraction is composed primarily of cell layers on the outside of the intact grains, which are rich in fiber constituents such as cellulose, hemicellulose, and lignin. In contrast to the endosperm, the cells within the bran are not disrupted to any significant degree during milling. The shorts fraction is composed of germ, which represents approximately 40% of the mass of this mill fraction, and residual bran and endosperm (*29*).

The bioavailability of Se from the different wheat mill fractions differed widely. Bioavailability relative to both NaSelenite and SeMet was almost 100% overall from flour, but only about 85% from wheat shorts. The lowest availability was only about 60% from wheat bran. The reason for the low availability from bran centers around the possibility that the bran proteins and other fibrous structures are not digested well in the upper gut. Wheat bran contains 45% nondigestible fiber, which is made up primarily of cell wall components. The low solubility of this fiber likely effectively encapsulates cellular proteins within intact cells in the bran fraction and renders them unavailable for digestion in the upper gut. Because most of the bran Se is incorporated primarily into these proteins, Se itself becomes unavailable. However, even though most of the bran fibers are transported to the colon where bacteria could digest them, the Se and selenoamino acids released apparently are not absorbed into the body to a great extent. More than likely, they are incorporated into bacterial proteins and excreted in the feces.

The transfer of Se to the colon in the indigestible fiber could be a positive outcome. Numerous studies have shown a reduced incidence of colon cancer in humans (*5*, *30*, *31*) and animals (*32*−*34*) who consumed Se in excess of the recommended normal intake. It is not known whether this benefit comes from Se that has been absorbed systemically or from that which has avoided digestion and is passed on to the colon. It has been shown that Se in the form of selenol compounds from broccoli are not absorbed as well as selenite or selenate (*35*), but are more effective in preventing precancerous lesions in the colon than the inorganic forms of Se (*36*). It is possible that the Se reaching the colon could be acted upon by the bacteria, which produce Se compounds that are effective in cancer prevention. However, this theory has not been explored.

Another possible scenario could be that Se along with the wheat bran is changing the bacterial populations of the colon to produce SCFAs, especially butyrate, which in turn could aid the colonocytes in resisting the formation of cancerous lesions. The indigestible fibers of wheat bran are known to produce high butyrate concentrations in the colon (*37*), and recent investigations in rats and mice strongly suggest that butyrate aids in the reduction of the incidence of cancerous lesions in this organ (*38*, *39*). However, investigations by Kim and Combs (*40*) do not lend support to this theory. When this group fed rats 13 times (2 mg of Se/kg) the Se requirement along with wheat bran (10%) to stimulate bacterial fermentation, they found that the concentrations of propionate and butyrate, as well as total SCFA, in the colon were actually reduced, compared with that in rats fed no Se. In the current study, the maximal amount of Se in the diet of rats fed 0.3% wheat bran was 0.05 mg of Se/kg. Whether this amount of Se would affect SCFA production by gut bacterial is not known.

Sodium selenite and SeMet are metabolized differently and accumulate at different rates in different pools in the rat (*41*). At dietary concentrations near that used in the current study, Whanger and Butler (*41*) found that Se from dietary SeMet was incorporated 2.8 times higher in muscle than NaSelenite and 1.3 times higher in brain. Our results were similar with 2 times difference for muscle and 1.3 time difference for brain. In both studies, there also was a small difference between NaSelenite and SeMet Se in liver with SeMet giving a higher value. This phenomenon is most likely caused by the fact that SeMet can

randomly substitute for methionine in tissue proteins. Beilstein and Whanger (*42*) showed that dietary Se supplied as SeMet accumulated more in hemoglobin and muscle than NaSelenite, but feeding NaSelenite resulted in Se being incorporated into selenoproteins primarily as selenocysteine. However, after an extended period, there was more Se as selenocysteine in liver than SeMet regardless of the starting material (*42*). It is recognized that the metabolic transformations of Se from various selenocompounds are tissue-specific and the deposition of Se from different dietary sources are seldom the same in all tissues.

Selenoprotein production and activity are dependent upon a constant and adequate supply of Se. In the current study, RBC Gpx1 and liver TRR activities reached maximal levels at a dietary concentration of about 80 μg/kg, whereas liver GPx1 did not reach maximal activity until around 140 μg/kg. Most studies show that Se from NaSelenite and SeMet are equally effective in restoring GPx1 activity (*43*, *44*); however, in another study by our group (*8*) we found that dietary Se from SeMet in excess of 100 μg/kg significantly elevated whole blood GPx1 activity by 10% over that when NaSelenite was fed. In the current experiment, however, this was not found when only RBC were used. Differences between these two Se sources with respect to stimulated enzyme activity also were observed by Xia et al. (*45*), who reported that plasma GPx3 activity was significantly greater in men fed SeMet than in those fed NaSelenite; however, upon extended feeding, the differences were less pronounced.

Bioavailability of Se from different fractions was assessed by evaluating the indicators of Se status in rats fed Se from the fractions relative to those to which SeMet or NaSelenite was fed. Three concentrations of Se from each wheat fraction were used, and the results showed that the responses for most parameters measured fell within the linear range produced by Se from NaSelenite and SeMet. One exception was testis Se, where the lowest level of Se from a wheat fraction maximized the amount of Se in this organ. This suggests that Se uptake and/or biochemical activity in the testes might be controlled by mechanisms different from those in other organs. However, work by Oldereid et al. (*46*) suggested that this was not the case in humans.

We pointed out in a previous publication (*8*) that determining bioavailability of Se from food by using a laboratory animal and then extrapolating this information to humans can be difficult. However, studies of this nature are starting points for evaluating food sources of Se with bioavailability potential high enough to be good natural Se supplements rather than resorting to the consumption of Se compounds in pill formulations. It was shown in this study that high Se from refined wheat flour, a common food ingredient in U.S. diets, could become an excellent source of highly bioavailable Se for Se supplementation.

ABBREVIATIONS USED

GPx1, glutathione peroxidase 1; NaSelenite, sodium selenite; NRC, National Research Council; SCFA, short chain fatty acids; SeMet, selenomethionine; TRR, thioredoxin reductase.

ACKNOWLEDGMENT

We acknowledge the assistance of Denice Schafer and staff for care of the animals and Lorna Hammerlund, Susan Fleck-Sheppard, and Deb Johnson for sample preparations and Se analysis.

LITERATURE CITED

(1) Levander, O. A. Selenium. In *Trace Elements in Human and Animal Nutrition*; Mertz, W. E., Ed.; Academic Press: New York, 1986; pp 209−279.

(2) Combs, G. F., Jr.; Lu, J. Selenium as a cancer preventative agent. In *Selenium. Its Molecular Biology and Role in Human Health*; Hatfield, D. L., Ed.; Kluwer Academic Publishers: Boston, MA, 2001; pp 205−219.

(3) Oldfield, J. E. A brief history of selenium research: from alkali disease to prostate cancer (from poison to prevention). Online only. American Society of Animal Science. http://www.asas.org/Bios/Oldfieldhist.pdf (2002).

(4) Duffield-Lillico, A. J.; Reid, M. E.; Turnbull, B. W.; Combs, G. F., Jr.; Slate, E. H.; Fischbach, L. A.; Marshall, J. R.; Clark, L. C. Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: a summary report of the Nutritional Prevention of Cancer Trial. *Cancer Epidemiol. Biomarkers Prev.* **2002**, *11*, 630−639.

(5) Clark, L. C.; Combs, G. F., Jr.; Turnbull, B. W.; Slate, E. H.; Chalker, D. K.; Chow, J.; Davis, L. S.; Glover, R. A.; Graham, G. F.; Gross, E. G.; Krongrad, A.; Lesher, J. L., Jr.; Park, H. K.; Sanders, B. B., Jr.; Smith, C. L.; Taylor, J. R. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. *JAMA−J. Am. Med. Assoc.* **1996**, *276*, 1957−1963.

(6) Hunt, J. R. Tailoring advice on dietary supplements: an opportunity for dietetics professionals. *J. Am. Diet. Assoc.* **2002**, *102*, 1754−1755.

(7) Levander, O. A. Considerations in the design of selenium bioavailability studies. *Fed. Proc.* **1983**, *42*, 1720−1725.

(8) Reeves, P. G.; Leary, P. D.; Gregoire, B. R.; Finley, J. W.; Lindlauf, J. E.; Johnson, L. K. Selenium bioavailability from buckwheat bran in rats fed a modified AIN-93G Torula yeast-based diet. *J. Nutr.* **2005**, *135*, 2627−2633.

(9) Gerrior, S.; Bente, L. *Nutrient Content of the U.S. Food Supply, 1909−99: A Summary Report*; USDA Center for Nutrition Policy and Promotion: Washingtin, DC, 2002.

(10) Moxon, A. L.; Olson, O. E.; Whitehead, E. I.; Hilmoe, R. J.; White, S. N. Selenium distribution in milled seleniferous wheats. *Cereal Chem.* **1943**, *20*, 376−380.

(11) National Research Council. *Guide for the Care and Use of Laboratory Animals*; Grossblatt, N., Ed.; National Academy Press: Washington, DC, 1996.

(12) Reeves, P. G.; Nielsen, F. H.; Fahey, G. C., Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **1993**, *123*, 1939−1951.

(13) National Research Council. *Nutrient Requirements of Laboratory Animals*, 4th revised ed.; Subcommittee on Laboratory Animal Nutrition; National Academy Press: Washington, DC, 1995.

(14) American Association of Cereal Chemists. International method 26-21.A. In *Approved Methods of the AACC*, 10th ed.; American Association of Cereal Chemists: St. Paul, MN, 2000; pp 1−5.

(15) Olson, O. E.; Novacek, E. J.; Whitehead, E. I.; Palmer, I. C. Investigations on selenium in wheat. *Phytochemistry* **1970**, *9*, 1181−1188.

(16) Wolf, W. R.; Goldschmidt, R. J. Selenomethionine contents of NIST wheat reference materials. *Anal. Bioanal. Chem.* **2004**, *378*, 1175−1181.

(17) Paglia, D.; Valentine, W. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* **1967**, *70*, 158−169.

(18) Hill, K. E.; McCollum, G. W.; Burk, R. F. Determination of thioredoxin reductase activity in rat liver supernatant. *Anal. Biochem.* **1997**, *253*, 123−125.

(19) Hintze, K. J.; Wald, K. A.; Zeng, H.; Jeffery, E. H.; Finley, J. W. Thioredoxin reductase in human hepatoma cells is transcriptionally regulated by sulforaphane and other electrophiles via an antioxidant response element. *J. Nutr.* **2003**, *133*, 2721−2727.

(20) Finney, D. J. *Statistical Method in Biological Assay*; Charles Griffin: London, U.K., 1978.

(21) Littell, R. C.; Henry, P. R.; Lewis, A. J.; Ammerman, C. B. Estimation of relative bioavailability of nutrients using SAS procedures. *J. Anim. Sci.* **1997**, *75*, 2672−2683.

(22) Mutanen, M.; Koivistoinen, P.; Morris, V. C.; Levander, O. A. Relative nutritional availability to rats of selenium in Finnish spring wheat (*Triticum aestivum* L.) fertilized or sprayed with sodium selenate and in an American winter bread wheat naturally high in Se. *Br. J. Nutr.* **1987**, *57*, 319−329.

(23) Shi, B.; Spallholz, J. Selenium from beef is highly bioavailable as assessed by liver glutathione peroxidase (EC 1.11.1.9) activity and tissue selenium. *Br. J. Nutr.* **1994**, *72*, 873−881.

(24) Smith, A. M.; Picciano, M. F. Relative bioavailability of selenocompounds in the lactating rat. *J. Nutr.* **1987**, *117*, 725−731.

(25) Cases, J.; Wysocka, I. A.; Caporiccio, B.; Joury, N.; Besançon, P.; Szpunar, J.; Rouanet, J. L. Assessment of selenium bioavailability from high-selenium spirulina subfractions in selenium-deficient rats. *J. Agric. Food Chem.* **2002**, *50*, 3867−3873.

(26) Finley, J. W. The absorption and tissue distribution of selenium from high-selenium broccoli are different from selenium from sodium selenite, sodium selenate, and selenomethionine as determined in selenium-deficient rats. *J. Agric. Food Chem.* **1998**, *46*, 3702−3707.

(27) Ørnsrud, R.; Lorentzen, M. Bioavailability of selenium from raw or cured selenomethionine-enriched fillets of Atlantic salmon (*Salmo salar*) assessed in selenium-deficient rats. *Br. J. Nutr.* **2002**, *87*, 13−20.

(28) Garrison, M. V.; Reid, R. L.; Fawley, P.; Breidenstein, C. P. Comparative digestibility of acid detergent fiber by laboratory albino and wild Polynesian rats. *J. Nutr.* **1978**, *108*, 191−195.

(29) Pomeranz, Y. Chemical composition of kernel sturctures. In *Wheat: Chemistry and Technology*; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1988; pp 97−158.

(30) Connelly-Frost, A.; Poole, C.; Satia, J. A.; Kupper, L. L.; Millikan, R. C.; Sandler, R. S. Selenium, apoptosis, and colorectal adenomas. *Cancer Epidemiol. Biomarkers Prev.* **2006**, *15*, 486−493.

(31) Jacobs, E. T.; Jiang, R.; Alberts, D. S.; Greenberg, E. R.; Gunter, E. W.; Karagas, M. R.; Lanza, E.; Ratnasinghe, L.; Reid, M. E.; Schatzkin, A.; Smith-Warner, S. A.; Wallace, K.; Martinez, M. E. Selenium and colorectal adenoma: results of a pooled analysis. *J. Natl. Cancer Inst.* **2004**, *96*, 1669−1675.

(32) Davis, C. D.; Feng, Y.; Hein, D. W.; Finley, J. W. The chemical form of selenium influences 3,2′-dimethyl-4-aminobiphenyl−DNA adduct formation in rat colon. *J. Nutr.* **1999**, *129*, 63−69.

(33) Feng, Y.; Finley, J. W.; Davis, C. D.; Becker, W. K.; Fretland, A. J.; Hein, D. W. Dietary selenium reduces the formation of aberrant crypts in rats administered 3,2′-dimethyl-4-aminobiphenyl. *Toxicol. Appl. Pharmacol.* **1999**, *157*, 36−42.

(34) Finley, J. W.; Davis, C. D.; Feng, Y. Selenium from high selenium broccoli protects rats from colon cancer. *J. Nutr.* **2000**, *130*, 2384−2389.

(35) Finley, J. W.; Grusak, M. A.; Keck, A. S.; Gregoire, B. R. Bioavailability of selenium from meat and broccoli as determined by retention and distribution of ^{75}Se. *Biol. Trace Elem. Res.* **2004**, *99*, 191−209.

(36) Finley, J. W.; Davis, C. D. Selenium (Se) arom high-selenium broccoli is utilized differently than selenite, selenate, and selenomethionine, but is more effective in inhibiting colon carcinogenesis. *BioFactors* **2001**, *14*, 191−196.

(37) Govers, M. J. A. P.; Gannon, N. J.; Dunshea, F. R.; Gibson, P. R.; Muir, J. G. Wheat bran affects the site of fermentation of resistant starch and luminal indexes related to colon cancer risk: a study in pigs. *Gut* **1999**, *45*, 840−847.

(38) Ohkawara, S.; Furuya, H.; Nagashima, K.; Asanuma, N.; Hino, T. Oral administration of *Butyrivibrio fibrisolvens*, a butyrate-producing bacterium, decreases the formation of aberrant crypt foci in the colon and rectum of mice. *J. Nutr.* **2005**, *135*, 2878−2883.

(39) Perrin, P.; Pierre, F.; Patry, Y.; Champ, M.; Berreur, M.; Pradal, G.; Bornet, F.; Meflah, K.; Menateau, J. Only fibers promoting a stable butyrate producing colonic ecosystem decrease the rate of aberrant crypt foci in rats. *Gut* **2001**, *48*, 53−61.

(40) Kim, J.; Combs, G. F., Jr. Effects of selenium on colonic fermentation in the rat. *Biol. Trace Elem. Res.* **1997**, *56*, 215−224.

(41) Whanger, P. D.; Butler, J. A. Effects of various dietary levels of selenium as selenite or selenomethionine on tissue selenium levels and glutathione peroxidase activity in rats. *J. Nutr.* **1988**, *118*, 846−852.

(42) Beilstein, M.; Whanger, P. D. Chemical forms of selenium in rat tissues after administration of selenite or selenomethionine. *J. Nutr.* **1986**, *116*, 1717−1719.

(43) Xia, Y.; Zhao, X.; Zhu, L.; Whanger, P. D. Metabolism of selenate and selenomethionine by a selenium-deficient population of men in China. *J. Nutr. Biochem.* **1992**, *3*, 202−210.

(44) Brown, K. M.; Pickard, K.; Nicol, F.; Beckett, G. J.; Duthie, G. G.; Arthur, J. R. Effects of organic and inorganic selenium supplementation on selenoenzyme activity in blood lymphocytes, granulocytes, platelets, and erythrocytes. *Clin. Sci.* **2000**, *98*, 593−599.

(45) Xia, Y.; Hill, K. E.; Byrne, D. W.; Xu, J.; Burk, R. F. Effectiveness of selenium supplements in a low-selenium area of China. *Am. J. Clin. Nutr.* **2005**, *81*, 829−834.

(46) Oldereid, N. B.; Thomassen, Y.; Purvis, K. Selenium in human male reproductive organs. *Hum. Reprod.* **1998**, *13*, 2172−2176.

Received September 7, 2006. Revised manuscript received November 14, 2006. Accepted November 15, 2006. This research was supported by the USDA, CSREES, Initiative for Future Agriculture and Food Systems (IFAFS) Grant 00-52102-9636, "High-Selenium Meat, Wheat, and Broccoli: A Marketable Asset", Dr. J. W. Finley, Principal Investigator, and by the USDA, CRIS Project 5450-51000-035-00D.

JF062572U