Selenium from beef is highly bioavailable as assessed by liver glutathione peroxidase (*EC* 1.11.1.9) activity and tissue selenium*

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The bioavailability of Se from ground beef has been previously found in this laboratory to be greater than that of selenite or selenate when fed to female Fischer 344 rats. In the present study we examined the bioavailability of Se from various commercial portions of beef, the liver, striploin, round, shoulder and brisket. All beef was cooked, freeze-dried, finely powdered and mixed with the other dietary ingredients. The experimental diets were fed to the weanling Fischer 344 rats which had been subjected to dietary depletion of Se for 6 weeks. The bioavailability of Se from the beef diets was compared with that of Se as selenite or L-selenomethionine (SeMet) added to torula-yeast diets. Each experimental diet contained 0·10 mg Se/kg. After 8 weeks of dietary Se repletion, relative activity of liver glutathione peroxidase (*EC* 1.11.1.9; GSHPx) from the different dietary groups compared with that of control animals (100 %) was (%): selenite 91, SeMet 122 ($P < 0.05$), liver 108, striploin 105, round 106, shoulder 106, brisket 103. Se recovery for liver GSHPx was generally highest from SeMet > beef muscle = beef liver > selenite. Muscle tissue deposition of Se was highest from SeMet > beef muscle > selenite = beef liver. In addition, the faecal excretion of Se was lowest from the SeMet dietary group and highest from the selenite dietary group. The experimental results suggest that all cuts of beef appear to be highly bioavailable sources of dietary Se when compared with selenite or L-SeMet.

Selenium bioavailability: Beef: Selenite: Selenomethionine

Se for people in the United States is mainly supplied by cereals, breads, meats and meat products (Schubert *et al.* 1987). Beef alone is estimated to contribute approximately 17 % of the total Se in the American diet (Holden *et al.* 1991).

The reported bioavailability of Se from foods varies considerably, ranging from 9 (dried fish solids) to 210 % (dehydrated lucerne (*Medicago sativa*) meal) when compared with Na_2SeO_3 (selenite; 100 %) in chickens (Cantor *et al.* 1981). In general, the bioavailability of Se from foods of plant origin is comparable with or somewhat greater than that of Na_2SeO_3. Se from foods of animal origin is generally less bioavailable (Combs & Combs, 1986). Such experimental conclusions have been based mainly on data from fish, chicken and meats other than beef in comparison with selenite and selenomethionine (SeMet; Douglass *et al.* 1981; Alexander *et al.* 1983; Bell & Cowey, 1989).

The bioavailability of Se from both raw and cooked ground beef has been previously found in this laboratory to be greater than that of Na_2SeO_3 or Na_2SeO_4 (selenate), as measured by recovery of liver glutathione peroxidase (*EC* 1.11.1.9; GSHPx) activity as well as by the total Se content deposited in liver and muscle of rats (Shi & Spallholz, 1994). In the present study we examined the bioavailability of Se from various portions of fully cooked commercial cuts of beef, including liver, striploin, round, shoulder and brisket. The

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bioavailability of Se from beef was compared with the bioavailability of Se from Na_2SeO_3 and L-SeMet in recovery experiments using weanling Fischer 344 rats.

MATERIALS AND METHODS

Animals

Female weanling Fischer 344 rats (n 210; Sasco/King Animal Laboratory, Omaha, NE, USA) were individually housed in suspended stainless-steel-wire cages in a temperature-controlled (21–24°) room with a 12 h light–dark cycle. Animals had free access to distilled water and diets.

Diets

A torula-yeast (TY) basal diet (Table 1) was used in this experiment. Various commercial portions of beef: the liver, striploin (loin), round, shoulder and brisket (from the ExCel Packing Plant, Plainview, TX, USA) were cooked well-done (approximately 3 h at 180° in a conventional General Electric home oven). They were then cut into small pieces, freeze-dried in a freeze-dryer (Labconco, Kansas City, MO, USA) for 24 h, finely powdered using a Waring blender, and analysed for their Se (Spallholz *et al.* 1978), crude fat, protein and moisture contents (Horwitz, 1970). To the TY basal diet was added 0·10 mg/kg of Se as Na_2SeO_3, L-SeMet, or Se as beef (Table 1). The fat and protein contents of all diets were adjusted to similar levels by adding or withholding beef tallow (fat), torula yeast (protein) or sucrose from the diets. The final mixed TY and beef diets were then assayed for their Se, crude fat and protein contents (Table 2). Food consumption for each dietary group of rats was measured throughout the 8-week repletion period.

Experimental design

The entire experiment lasted 14 weeks with two distinguishable periods. The Se-depletion and Se-repletion periods consisted of 6 and 8 weeks respectively. For the first 6 weeks (Se-depletion period) the animals were divided into two dietary groups. Thirty animals were fed on the TY diet to which had been added 0·10 mg Se as Na_2SeO_3/kg (control group). The remaining animals were all fed on the TY basal diet which contained 0·008 (SD 0·0002) mg Se/kg (Se-deficient group). At the end of the 6-week depletion period six animals from each of the two groups were killed and tissues (liver and muscle) were removed for Se bioassays. From week 7 to week 14 (repletion period) the animals were divided into eight dietary groups. Each separate diet contained about 0·10 mg Se as Na_2SeO_3/kg (Table 2), SeMet, beef liver, striploin, round, shoulder or brisket. Six animals from each of the eight dietary groups were killed at weeks 1, 2, 4 and 8 of the repletion period. Liver and muscle tissues obtained from each animal were then assayed for their total Se content. Liver tissue was analysed for GSHPx activity.

Selenium bioassays

The rats were weighed and killed by cervical dislocation. Liver (medulla lobe) and muscle (left posterior leg) tissues were excised, washed in cold saline (9 g NaCl/l), blotted, weighed and stored at $+4°$. Within 12 h liver tissues were homogenized in 3 vol. of cold sucrose solution (0·25 mol/l) using a Potter-Elvehjem mortar and pestle and were centrifuged using a Beckman J-21 ultracentrifuge (Palo Alto, CA, USA) for 20 min at 35000 g at $+4°$. The tissue supernatant fractions were collected and kept frozen at $-75°$ until assayed for the Se-dependent GSHPx and protein. GSHPx activity was assayed by a modification of the method of Paglia & Valentine (1967) using a u.v.–visible model UV-160 spectrophotometer (Shimadzu, Kyoto, Japan) by monitoring the oxidation of NADPH at 340 nm. Our modified cocktail mixture contained (mmol/l): glutathione 2, NADPH 0·15, glutathione reductase (*EC* 1.6.4.2) 1 unit, NaN_3 1, potassium phosphate buffer (pH 7·0) 50 and EDTA 1·5.

Table 1. *Composition of experimental diets (g/kg)*

		Beef diets				
Ingredients	TY diet*	Liver	Striploin	Round	Shoulder	Brisket
Torula yeast	200	155	104	91	86	43
Beef	0.0	45	127	125	143	208
Sucrose	645	645	645	645	645	634
Beef tallow	40	40	9	24	11	0·0
Maize oil	10	10	10	10	10	10
Others†	155	155	155	155	155	155

* Protein source is torula yeast.

† All diets contained the same amounts of the following five components (g/kg): AIN Mineral Mixture 76 omitting Se 40, AIN Vitamin Mixture 76 10, cellulose 50, choline bitartrate 0·2, DL-methionine 0·3. All these five chemicals were purchased from the United State Biochemical Corporation, Cleveland, OH, USA.

Table 2. *Selenium, crude fat and protein concentrations, energy and moisture in experimental diets**

(Mean values and standard deviations for four samples)

	Se† (mg/kg)		Fat‡ (g/kg)	Protein‡ (g/kg)	Energy§ (KJ/kg)	Moisture‡ (g/kg)
Diets	Mean	SD				
Control	0·115	0·020	57	99	14660	57
Selenite	0·115	0·020	57	99	14660	57
SeMet	0·115	0·013	57	99	14660	57
Liver	0·109	0·008	63	104	14950	63
Striploin	0·117	0·006	56	139	15290	56
Round	0·117	0·011	57	142	15370	57
Shoulder	0·112	0·011	55	148	15410	55
Brisket	0·114	0·006	68	151	15750	68

SeMet, selenomethionine.

* For details of diets, see Table 1 and p. 874.

† Assayed by the fluorometric method of Spallholz *et al.* (1978).

‡ Crude fat, protein and moisture contents of diets were measured by the proximate analysis of Horwitz (1970).

§ Calculated by (g crude fat × 37·8) + (g crude protein (N × 6·25) × 16·8) + (g sucrose × 16·8).

H_2O_2 (0·25 mmol/l) was used as the substrate for GSHPx. The protein content of the tissue homogenates was determined by a commercial protein assay (Bio-Rad Laboratories, Richmond, CA, USA; Simpson & Sonne, 1982). Protein standards were prepared from bovine serum albumin (Sigma, St Louis, MO, USA). About 100 mg liver tissue, 300 mg muscle and 150 mg faeces were collected from each animal for total Se concentration analysis by the fluorometric method described by Spallholz *et al.* (1978) using a fluorescence spectrophotometer (model 650-40; Perkin-Elmer, Norwalk, CT, USA). Standard curves were prepared using Na_2SeO_3.

Statistical analysis

All values are given as the mean values and standard deviation. Statistical differences between means were determined by one-way ANOVA and the level of significance was set at $P < 0.05$.

Table 3. *Liver glutathione peroxidase* (EC *1.11.1.9*; *GSHPx*; *mU/min per mg protein*) *activity of rats during the selenium-depletion and Se-repletion periods**

(Mean values and standard deviations for six animals)

Treatment group	Period of depletion (weeks)				Period of repletion (weeks)							
	0		6		1		2		4		8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	333	51†	726ᵃ	44‡	866ᵃ	70	665ᵃ	51	669ᵃ	67	669ᵇᶜ	24
Se-deficient			14ᵇ	2§								
Selenite					135ᶜ	33	294ᵉᶠ	45	573ᵇ	42	608ᶜ	32
SeMet					233ᵇ	22	542ᵇ	44	714ᵃ	61	817ᵃ	36
Liver					115ᶜ	19	304ᵈᵉ	27	595ᵇ	71	723ᵇ	90
Striploin					96ᶜ	16	258ᶠ	19	564ᵇ	53	703ᵇ	71
Round					108ᶜ	17	362ᶜ	22	584ᵇ	22	706ᵇ	49
Shoulder					112ᶜ	16	342ᶜᵈ	34	601ᵇ	19	710ᵇ	91
Brisket					96ᶜ	14	296ᵉᶠ	38	551ᵇ	25	691ᵇ	80

[a, b, c, d, e, f] Mean values in a column with different superscript letters were significantly different (one-way ANOVA; $P < 0.05$).

SeMet, selenomethionine.

* For details of diets and treatments, see Table 1 and p. 876.

† Value from six animals fed on a normal rodent diet on arrival.

‡ Value from six animals fed on a Se-adequate (control) torula-yeast diet for 6 weeks.

§ Value from six animals fed on a Se-deficient diet for 6 weeks.

RESULTS

Feed consumption and body weights of rats

Feed consumption among all dietary groups of rats was similar during the repletion period. No significant differences in the body weights between groups of rats were observed at the end of the experimental period. Rats fed on the beef diets were no more than 5·5 % heavier than the animals fed on the TY diets during the entire Se-repletion period.

Liver glutathione peroxidase activity

Weanling rats fed on the Se-deficient TY basal diet for 6 weeks had only 1·9 % of the liver GSHPx activity of the control animals arbitrarily set at 100 % activity. During the Se-repletion period liver GSHPx activity recovered rapidly with the highest recovery occurring in the dietary SeMet group of animals. The rats fed on selenite had a recovery of GSHPx activity similar to the rats fed on beef during the first 4 weeks of the repletion period. These selenite-fed animals showed a significantly lower GSHPx activity than all beef dietary groups by week 8 of the repletion period ($P < 0.05$, Table 3).

Selenium deposition in tissues

At 6 weeks, Se-deficient rats had only 11 % Se in the liver and 47 % Se in muscle tissue compared with control animals whose Se tissue concentrations were arbitrarily set at 100 %. During the Se-repletion period both liver and muscle tissues recovered their Se concentration. The SeMet group recovered their Se deposition in liver and muscle tissues faster than any other dietary group. The recovery of liver Se among the beef dietary groups and the selenite group was similar. All beef-muscle dietary groups of rats had a slightly higher muscle Se concentration than the beef-liver dietary group and the selenite group (Tables 4, 5). Muscle Se in animals fed on the liver diet was only 90 % of the control by the

Table 4. *Total liver selenium concentration (ng/g wet weight) of rats during the Se-depletion and Se-repletion periods**

(Mean values and standard deviations for six animals)

Treatment group	Period of depletion (weeks)				Period of repletion (weeks)							
	0		6		1		2		4		8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	1054	192†	936^a	195‡	1084^a	232	1010^a	100	1064^a	141	1006^c	105
Se-deficient			107^b	12§								
Selenite					204^d	86	558^e	19	930^a	93	1059^{bc}	60
SeMet					532^b	74	926^b	76	1069^a	60	1229^a	129
Liver					332^e	36	516^a	80	942^a	77	1029^{bc}	132
Striploin					343^e	25	617^d	34	965^a	116	1063^{bc}	112
Round					372^e	22	732^c	29	1027^a	80	1114^{abc}	46
Shoulder					361^c	30	575de	53	956^a	28	1176^{ab}	145
Brisket					317^e	38	545de	59	995^a	176	1009^c	162

^{a,b,c,d,e} Mean values in a column with different superscript letters were significantly different (one-way ANOVA; $P < 0.05$).

SeMet, selenomethionine.

* For details of diets and treatments, see Table 1 and p. 876.

† Value from six animals fed on a normal rodent diet on arrival.

‡ Value from six animals fed on a Se-adequate (control) torula-yeast diet for 6 weeks.

§ Value from six animals fed on a Se-deficient diet for 6 weeks.

Table 5. *Total muscle selenium concentration (ng/g wet weight) of rats during the Se-depletion and Se-repletion periods**

(Mean values and standard deviations for six animals)

Treatment group	Period of depletion (weeks)				Period of repletion (weeks)							
	0		6		1		2		4		8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	125	24†	109^a	10†	117^a	10	112^a	8	105^a	11	109^c	15
Se-deficient			51^b	7†								
Selenite					58^d	4	58^e	6	73^b	7	102^d	15
SeMet					72^b	3	90^b	11	117^a	11	152^a	16
Liver					58^d	5	63de	9	81^b	10	98^d	22
Striploin					63^{cd}	3	77^c	15	105^a	26	118^{bcd}	7
Round					60^{cd}	5	77^c	10	110^a	16	125^{bc}	13
Shoulder					67^{bc}	5	70^{cd}	10	80^b	15	137^{ab}	18
Brisket					57^d	9	71^{cd}	5	83^{ab}	11	115^{cd}	14

^{a,b,c,d,e} Means in a column with different superscript letters were significantly different (one-way ANOVA; $P < 0.05$).

SeMet, selenomethionine.

* For details of diets and treatments, see Table 1 and p. 876.

† Value from six animals fed on a normal rodent diet on arrival.

‡ Value from six animals fed on a Se-adequate (control) TY diet for 6 weeks.

§ Value from six animals fed on a Se-deficient diet for 6 weeks.

Table 6. *Total faecal selenium concentration (g/kg dry weight) of rats during the Se-depletion and Se-repletion periods**

(Mean values and standard deviations for six animals)

Treatment group	Period of depletion (weeks)						Period of repletion (weeks)							
	0		3		6		1		2		4		8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	423	46†	188ᵃ	35‡	236ᵃ‡	38‡	338ᵃ	65	308ᵃ	34	296ᵃ	8	308ᵃ	15
Se-deficient			40ᵇ	3§	30ᵇ	4§								
Selenite							252ᵇ	27	271ᵃ	28	258ᵃᵇ	30	302ᵃᵇ	28
SeMet							114ᵉ	8	197ᵇ	21	175ᶜ	23	188ᵈ	21
Liver							208ᶜ	28	272ᵃ	46	244ᵇ	32	264ᵃᵇᶜ	32
Striploin							176ᶜᵈ	8	147ᶜ	20	221ᵇ	35	261ᵃᵇᶜ	37
Round							149ᵈᵉ	21	203ᵃ	37	219ᵇ	44	219ᶜᵈ	44
Shoulder							171ᶜᵈ	20	212ᵇ	7	229ᵇ	25	247ᵇᶜ	50
Brisket							183ᶜᵈ	5	215ᵇ	27	244ᵇ	18	247ᵇᶜ	40

$^{a, b, c, d, e}$ Means in a column with different superscript letters were significantly different (one-way ANOVA; $P < 0.05$).

SeMet, selenomethionine.

* For details of diets and treatments, see Table 1 and p. 876.

† Value from six animals fed on a normal rodent diet on arrival.

‡ Value from six animals fed on a Se-adequate (control) torula-yeast diet for 6 weeks.

§ Value from six animals fed on a Se-deficient diet for 3 or 6 weeks respectively.

end of the repletion period while all animals fed on beef diets surpassed the control group in the recovery of their muscle Se.

Faecal selenium concentration

Faecal Se decreased rapidly during the Se-depletion period and increased sharply when dietary Se was resupplemented to rats. Faecal Se levels recovered to control levels within the first 2 weeks of the repletion period in all dietary groups. Faecal Se was lowest in the SeMet-fed animals and highest in the selenite-fed animals. The animals fed on beef liver had the highest level of faecal Se among all dietary groups of rats fed on beef diets (Table 6).

DISCUSSION

The literature on the bioavailability of Se from animal foods such as fish and meat has generally held the premise that this Se is not as highly bioavailable when compared with equivalent amounts of dietary selenite. Se from plant foods, on the other hand, has been recognized as being more highly bioavailable than Se from animal foods or dietary selenite (Combs & Combs, 1986).

This laboratory has recently shown that the Se from either raw or cooked ground beef is similar or slightly more bioavailable when compared with dietary selenite fed to Fischer 344 rats (Shi & Spallholz, 1994). In the present study, conducted in a similar manner to our previous study using raw and cooked ground beef, a comparison was made between the bioavailability of Se from commercial portions of beef and selenite or L-SeMet. L-SeMet was used as a reference source of dietary Se because it is the major dietary form of Se in cereal grains (Osman & Latshaw, 1976; Sathe *et al.* 1992). In addition, our previous experience with the ground-beef diets suggested to us that SeMet may be a significant source of dietary Se, not only in cereal grains but also in beef (Shi & Spallholz, 1994). This inference was gleaned from our previous findings showing that dietary Se repletion in the muscle of Fischer 344 rats was higher from beef diets than from Na_2SeO_3.

It is very well known that dietary SeMet will accumulate in muscle tissues to a greater extent than selenite or selenate (Martin & Hurlbut, 1976; Deagen *et al.* 1987; Behne *et al.* 1991). This occurs because SeMet is incorporated into and retained by the primary muscle protein structure replacing methionine whereas dietary selenite and selenate are not retained in the primary protein of muscle (Martin & Hurlbut, 1976; Deagen *et al.* 1987; Salbe & Levander, 1990) nor are these inorganic Se compounds converted to SeMet in animals (Young *et al.* 1982). Accumulation of Se in muscle tissue is a distinct marker for dietary SeMet occurring in the rat (Osman & Latshaw, 1976; Behne *et al.* 1991; Vendeland *et al.* 1991).

Se bioavailability can be assessed by: (1) the 'functional assay' of GSHPx activity, (2) 'tissue residue level' of Se, or by (3) prevention of a Se-deficiency disease (Combs & Combs, 1986). Of these three approaches of mineral bioavailability, the third is the least preferred. Because liver GSHPx activity is very sensitive to both Se depletion and Se repletion (Shi & Spallholz, 1994), and because liver and skeletal muscle comprise the greatest body stores of Se, approximately 30 and 40% respectively (Behne & Wolters, 1983), liver GSHPx activity as well as liver and muscle Se concentration were employed as the criteria of the bioavailability of Se in the present study.

Recovery from the depletion of liver GSHPx by rats fed on L-SeMet and beef diets was greater than from the animals fed on selenite at week 8 of the experimental recovery period. Also at week 8, liver GSHPx in comparison with control animals fed on selenite (100%) was: SeMet (122%, $P < 0.05$), striploin (105%), round (106%), shoulder (106%), brisket

(103%), liver (108%) and selenite (91%). Thus, all beef diets restored liver GSHPx to recovery values intermediate between those of animals fed on L-SeMet and selenite. GSHPx recovery from depletion of this liver enzyme in rats was the primary means of assessing Se bioavailability (Combs & Combs, 1986; Greger, 1992).

Total tissue levels of Se in liver and muscle tissues are secondary measures of bioavailability for dietary Se. Once again, L-SeMet-fed rats recovered more Se both in liver and muscle than the rats fed on the commercial beef diets or the selenite-containing diet. Animals fed on beef-muscle diets recovered their muscle Se more rapidly than animals fed on beef liver and those consuming the selenite diet. The recovery of liver Se showed no significant difference among animals fed on beef diets and those fed on the selenite diet during the later stage (weeks of 4 and 8) of the recovery period. We also observed that animals fed on selenite had a higher faecal Se content than those fed on either SeMet or beef Se. Thus, dietary Se absorption constitutes part of the reason for the higher bioavailability of SeMet and beef Se than that of selenite.

The bioavailability of dietary Se is affected not only by its chemical form (Combs & Combs, 1986) but also by other dietary factors such as the total protein and fat contents of the diet (Osman & Latshaw, 1976; Zhou et al. 1983; Smith & Picciano, 1987). The experiment here was designed to be both isoenergetic and nearly isonitrogenous within the experimental limits imposed in formulating 0·10 mg Se/kg diets from beef. In addition, animals depleted of Se and repleted with diets containing equal amounts of Se as selenite, L-SeMet or the commercial cooked beef consumed the same amount of diet as provided ad lib. Animals consuming the beef diets were not significantly larger ($P > 0·05$) in body weight than the animals consuming the Se-supplemented TY diets at the end of the repletion period.

Thus, we conclude from our previous study of raw and cooked ground beef (Shi & Spallholz, 1994) and the present study of the bioavailability of Se from various cuts of commercial beef, that the bioavailability of Se from beef is higher than or at least equal to that of selenite and slightly lower than that of L-SeMet. Our results here are consistent with two recent studies in humans which showed that meat was as good a source of Se, as is wheat (van der Torre et al. 1991), and that SeMet was absorbed more rapidly than selenite in Se-deficient men (Xia et al. 1992).

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