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Specific Activity of Methionine Sulfoxide Reductase in CD-1 Mice Is Significantly Affected by Dietary Selenium But Not Zinc

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ABSTRACT

Reactive oxygen species-mediated oxidation of methionine residues in protein results in a racemic mixture of R and S forms of methionine sulfoxide (MetO). MetO is reduced back to methionine by the methionine sulfoxide reductases MsrA and MsrB. MsrA is specific toward the S form and MsrB is specific toward the R form of MetO. MsrB is a selenoprotein reported to contain zinc (Zn). To determine the effects of dietary selenium (Se) and Zn on Msr activity, CD-1 mice (N=16/group) were fed, in a 2×2 design, diets containing 0 or 0.2 µg Se/g and 3 or 15 µg Zn/g. As an oxidative stress, half of the mice received L-buthionine sulfoximine (BSO; ip; 2 mmol/kg, three times per week for the last 3 wk); the others received saline. After 9.5 wk, Msr (the combined specific activities of MsrA and MsrB) was measured in the brain, kidney, and liver. Se deficiency decreased (p<0.0001) Msr in all three tissues, but Zn had no direct effect. BSO treatment was expected to result in increased Msr activity; this was not seen. Additionally, we found that the ratio of MetO to methionine in liver protein was increased (indicative of oxidative damage) by Se deficiency. The results show that Se deficiency increases oxidation of methionyl residues in protein, that Se status affects Msr (most likely through effects on the selenoprotein MsrB), and that marginal Zn deficiency has little effect on Msr in liver and kidney. Finally, the results show that the oxidative effects of limited BSO treatment did not upregulate Msr activity.

Index Entries: Methionine sulfoxide reductase; mice; oxidation; selenium deficiency; zinc.

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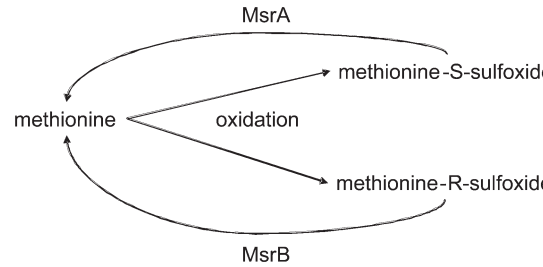

Fig. 1. Oxidation of methionine to methionine-*S*-sulfoxide or methionine-*R*-sulfoxide and reduction back to methionine by MsrA or MsrB, respectively.

INTRODUCTION

The oxidation of protein-bound methionyl residues to the corresponding methionine sulfoxide (MetO) can lead to loss of biological activity in a variety of proteins *(1–3).* This loss of activity can be reversed by the methionine sulfoxide reductases MsrA and MsrB, which catalyze the reduction of MetO back to methionine (Fig. 1). MsrA has been known for over four decades *(4).* However, it was shown that MsrA cannot account for all Msr activity and that MsrA is stereo-specific for methionine-*S*-sulfoxide *(5).* The nonenzymatic oxidation of methionine produces both methionine-*S*-sulfoxide and methionine-*R*-sulfoxide. MsrB, which is stereo-specific for methionine-*R*-sulfoxide, has only recently been discovered *(6,7).* Furthermore, MsrB was found to be a selenoprotein *(6,8)* that also contains 1.08 equivalents of zinc (Zn) *(7).* The Zn appears to be stably associated with the protein, as significant Zn remains after extensive dialysis either in the presence of absence of EDTA *(7).* Thus, both MsrA and MsrB are important for antioxidant defense. Furthermore, one hypothesis suggests that free and protein-bound methionine act as scavengers for reactive oxygen species (ROS) *(3);* that is, oxidation of methionine residues to MetO on the surface (away from the active site) of many proteins results in elimination of ROS without loss of biological activity of the respective protein. Therefore, it appears that ROS might be eliminated by free methionine and cellular methionine-containing proteins as long as the Msr system is functioning properly.

The fact that certain proteins can be inactivated by the oxidation of a single methionyl residue suggests that the Msr system might function in the regulation of such proteins *(1,9).* A very important aspect of this system is that both MsrA and MsrB require thioredoxin and the selenium (Se) dependent enzyme thioredoxin reductase to provide the reducing equivalents necessary for reduction of MetO to methionine *(5,10).*

The present study was designed to determine the effects of dietary Se and Zn on the activity of the Msr system. In addition, buthionine sulfoximine (BSO) was included as a treatment to induce oxidant stress (inhibits

Table 1
Basal Diet

Ingredient	g/kg diet
Amino acid mix[1]	168.7
Sucrose	509.3
Soybean oil	70.0
Mineral mix[2]	20.0
Vitamin mix[3]	5.0
Alphacel fiber	50.0
Choline bitartrate	2.5
Corn starch	150.0
$CaCO_3$	7.0
KH_2PO_4	10.0
$CaHPO_4$	7.5
Total:	1000.0

[1] Amino acid mix, in g/kg diet: L-arginine, 7.5; L-histidine HCl· H_2O, 6.0; L-lysine HCl, 16.5; L-tyrosine, 7.5; L-tryptophan, 2.5; L-phenylalanine, 7.5; L-threonine, 7.5; L-leucine, 11.0; L-isoleucine, 9.0; L-valine, 10.5; glycine, 6.0; L-proline, 4.5; L-glutamic acid, 40.0; L-alanine, 4.0; L-serine, 4.0; L-aspartic acid, 7.0; L-asparagine, 6.0; L-methionine, 8.2; L-cystine, 3.5.

[2] Mineral mix, in g/kg diet: K_2SO_4, 1.632; $CaCl_2 \cdot 2H_2O$, 1.05; Na_2CO_3, 1.5; MgO, 0.85; $Na_2SiO_3 \cdot 9H_2O$, 0.254; ferric citrate (16% Fe), 0.22; manganese carbonate (43% Mn), 0.024; cupric carbonate (55% Cu), 0.011; sucrose, 14.44; in mg/kg diet: KIO_3, 0.35; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.371; $CrK(SO_4)_2 \cdot 12H_2O$, 9.625; H_3BO_3, 2.853; NaF, 2.223; nickel carbonate, 2.223; SnO, 0.25; NH_4VO_3, 0.462; $NaAsO_2$, 0.4.

[3] Vitamin mix, in mg/kg diet: nicotinic acid, 30; pantothenic acid, Ca salt, 16; pyridoxine HCl, 7; thiamine HCl, 6; riboflavin, 6; folic acid, 2; D-biotin, 0.2; vitamin B_{12} in 0.1% mannitol, 25; DL-α-tocopherol acetate (300 IU/g), 250; retinyl palmitate (250,000 IU/g), 16; vitamin D_3 (400,000 IU/g), 2.5; vitamin K_1, 1; sucrose, 4638.

γ-glutamylcysteine synthetase) for determining whether limited exposure to this compound affected Msr activity.

MATERIALS AND METHODS

Animals and Diets

Male, weanling CD-1 mice (Charles River Laboratories, Wilmington, MA) were weighed upon arrival and assigned to each dietary group (N=16 per group) with no statistical difference in initial weight among groups.

Mice were housed in groups of four in a room with controlled temperature and light. Mice were provided free access to demineralized water and purified diet. The amino-acid-based diet (Table 1) was supplemented with 0 or 0.2 µg Se (as selenite)/g and 3 or 15 µg Zn (as zinc carbonate)/g. The Se and Zn requirements for the mouse are estimated to be 0.15 µg Se/g and 10 µg Zn/g *(11).* The basal amino-acid-based diet was based on AIN-93 recommendations *(12,13)* and by analysis contained approx 3 ng Se/g and approx 0.5 µg Zn/g. The mice were fed their respective diets for 9.5 wk. During the last 3 wk of feeding, one-half of the mice (*N*=8) from each group were injected (ip) three times per week with 2 mmol L-buthionine sulfoximine kg body weight/(Sigma, St. Louis, MO); the other eight mice from each group were injected with the 0.9% saline vehicle. At the end of wk 9.5, the mice were anesthetized with xylazine (Rompon, Moboay Inc., Shawnee, KS) and ketamine (Ketaset, Aveco Inc., Fort Dodge, IA) and killed by exsanguination. This study was approved by the Animal Care Committee of the Grand Forks Human Nutrition Research Center, and the mice were maintained in accordance with the guidelines for the care and use of laboratory animals.

Plasma Homocysteine, Cysteine, and Glutathione

Total (reduced plus oxidized) homocysteine, total cysteine, and total glutathione were determined in heparinized plasma by using high-performance liquid chromatography (HPLC) according to the procedure of Durand et al. *(14).*

Selenium Status

Glutathione peroxidase (GPx) enzyme activity was determined by the coupled enzymatic method of Paglia and Valentine *(15),* which uses hydrogen peroxide (0.15 µ*M*) as the substrate. Thioredoxin reductase (TRR) activity was determined according to Hintze et al. *(16).*

Methionine Sulfoxide Reductase

Tissue-specific activity of Msr was determined by the method of Moskovitz and Stadtman *(17).* In this assay, the reduction of protein-bound MetO by Msr was assayed by using dabsyl-*R,S*-methionine sulfoxide as substrate. The substrate was made by derivitization of L-methionine-*R,S*-sulfoxide with dabsyl chloride (4-*N,N*-dimethylaminoazobenzene- 4′-sulfonyl chloride) according to Moskovitz et al. *(18).* Because the substrate was a racemic mixture, the assay does not differentiate between MsrA and MsrB.

Protein Methionine Sulfoxide

Methionine sulfoxide in liver protein was determined by the method of Maier et al. *(19).* The method is based on cyanogen bromide cleavage of the carboxyl-side peptide of methionine to yield homoserine. However,

Table 2
Effect of Dietary Se, Zn, and BSO
on Body Weight, Liver GPx, and TRR

Treatment[1]	Body Weight, g	Liver GPx u/mg protein	Liver TRR u/mg protein
0 Se 3 Zn Saline	41.8±4.1[2]	14.1±12.6	0.041±0.012
0 Se 3 Zn BSO	39.1±3.5	22.5±13.2	0.040±0.009
0 Se 15 Zn Saline	41.1±3.3	21.5±17.2	0.057±0.024
0 Se 15 Zn BSO	39.8±3.5	27.8±21.0	0.040±0.017
0.2 Se 3 Zn Saline	43.1±3.2	1220±99	0.169±0.022
0.2 Se 3 Zn BSO	43.3±3.3	1222±178	0.184±0.034
0.2 Se 15 Zn Saline	41.1±6.5	1158±201	0.162±0.021
0.2 Se 15 Zn BSO	40.9±4.8	1047±124	0.148±0.041
Analysis of variance, p values			
Se	NS[3]	0.0001	0.0001
Zn	NS	NS	NS
Se x Zn	NS	0.035	0.023
BSO/Saline	NS	NS	NS
Se x BSO/Saline	NS	NS	NS
Zn x BSO/Saline	NS	NS	NS
Se x Zn x BSO/Saline	NS	NS	NS

[1] Values for Se and Zn are in µg/g diet. BSO/saline: One-half of the mice (N=8) from each group were injected (ip) three times per week with 2 mmol L-buthionine sulfoximine/kg body weight; the other eight mice from each group were injected with the 0.9% saline vehicle.
[2] Values are means ± SD, N=8 per group.
[3] NS=not significant (p>0.05).

cyanogen bromide does not cleave the bond if the methionine residue is oxidized to methionine sulfoxide. After cyanogen bromide treatment, the resultant peptides were hydrolyzed to individual amino acids by the method of Schilling et al. *(20)* with the addition of 1% phenol to the *6 N* HCl. Following hydrolysis, amino acid analysis was carried out by the method of Turnell and Cooper *(21).* Duplicate samples that were not treated with cyanogen bromide were carried through the procedure.

Statistical Analyses

Although the mice were housed in groups of four, it was assumed that the animals within each cage are no more or no less alike than animals in different cages. Hence, the animal in each cage, rather than the cage itself, was used as the sampling unit. The data were analyzed by a three-way

Table 3
Effect of Dietary Se, Zn, and BSO Plasma Homocysteine (Hcy), Cysteine, and GSH

	Plasma		
	Hcy	Cysteine	GSH
Treatment[1]		μmol/L	
0 Se 3 Zn Saline	2.22±0.65[2]	75.7±8.4	20.2±5.5
0 Se 3 Zn BSO	2.60±0.37	85.6±13.8	20.7±3.9
0 Se 15 Zn Saline	2.78±0.99	87.5±14.2	21.7±7.4
0 Se 15 Zn BSO	2.17±0.57	80.1±9.9	19.1±3.9
0.2 Se 3 Zn Saline	4.87±1.27	125±22.7	21.2±7.9
0.2 Se 3 Zn BSO	4.29±1.20	123±20.3	18.7±2.9
0.2 Se 15 Zn Saline	3.78±2.06	111±26.8	19.0±4.6
0.2 Se 15 Zn BSO	2.97±0.52	92.2±10.1	16.2±3.6
Analysis of variance, p values			
Se	0.0001	0.0001	NS[3]
Zn	0.046	0.033	NS
Se x Zn	0.026	0.005	NS
BSO/Saline	NS	NS	NS
Se x BSO/Saline	NS	NS	NS
Zn x BSO/Saline	NS	NS	NS
Se x Zn x BSO/Saline	NS	NS	NS

[1] Values for Se and Zn are in μg/g diet. BSO/saline: One-half of the mice (N=8) from each group were injected (ip) three times per week with 2 mmol L-buthionine sulfoximine/kg body weight; the other eight mice from each group were injected with the 0.9% saline vehicle.
[2] Values are means ± SD, N=8 per group.
[3] NS=not significant (p>0.05).

analysis of variance (ANOVA) using the SAS general linear models program (SAS Version 9.1; SAS Institute, Cary, NC). Tukey's contrasts were used to differentiate among means for variables significantly (p<0.05) affected by Se, Zn, BSO/saline, or their interactions. For effects of Se on liver protein MetO/Met ratio, the t-test was used to compare values, with p<0.05 considered significant. All values are reported as means ± SD.

RESULTS

Table 2 shows that growth over the 9.5 wk of the experiment was not affected by diet (Se or Zn) or by BSO/saline treatment. The Se status indi-

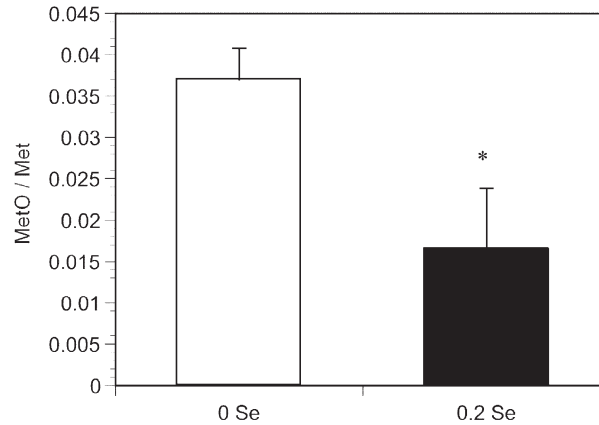

Fig. 2. The effect of dietary Se on the ratio of MetO to methionine (Met) in liver protein. Values for Se are in μg/g diet. Bars represent means ± SD, N=4 per group. Only saline-treated animals were used, with marginal and adequate dietary Zn equally represented at each Se level. *Significant effect (as determined by the t-test), $p<0.05$.

cators, liver GPx and TRR, were markedly reduced ($p<0.0001$) in mice fed the Se-deficient diet. Se interacted with Zn to similarly affect GPx (p=0.035) and TRR (p=0.023) activities. When Se was adequate, Zn supplementation decreased the activities of GPx and TRR. However, when Se was low, Zn supplementation had no effect on the activities.

Plasma homocysteine and cysteine were significantly decreased by Se deprivation ($p<0.0001$) (Table 3). Se supplementation to the mice fed the marginal Zn diet resulted in an increase in plasma homocysteine; there was no effect on homocysteine by Se supplementation of mice fed the Zn-adequate diet (Se × Zn, p=0.026). Zn supplementation to mice fed the Se-adequate diet resulted in a decrease in plasma cysteine; there was no effect of Zn supplementation on cysteine in the mice fed low Se (Se × Zn, p=0.005). Dietary Se or Zn or BSO/saline treatment did not significantly affect plasma GSH.

The effect of dietary Se on the ratio of MetO/methionine in liver protein is shown in Fig. 2. The ratio of MetO/methionine is increased approx 2.2-fold by Se deficiency ($p<0.05$). Limited samples were used for this assay: Only saline-treated animals were used, with marginal and adequate dietary Zn equally represented at each Se level.

Figures 3–5 show the effect of dietary Se and Zn and of BSO/saline on the liver, kidney, and brain Msr activity, respectively. In each tissue, Msr activity is significantly reduced by Se deficiency ($p<0.0001$). Se deficiency reduced Msr activity to the greatest extent in the liver (83% reduction) followed by the kidney (63% reduction) and then the brain (25% reduction). Msr activity in the liver was affected by an interaction between Zn and BSO/saline (p= 0.035; Fig. 3).

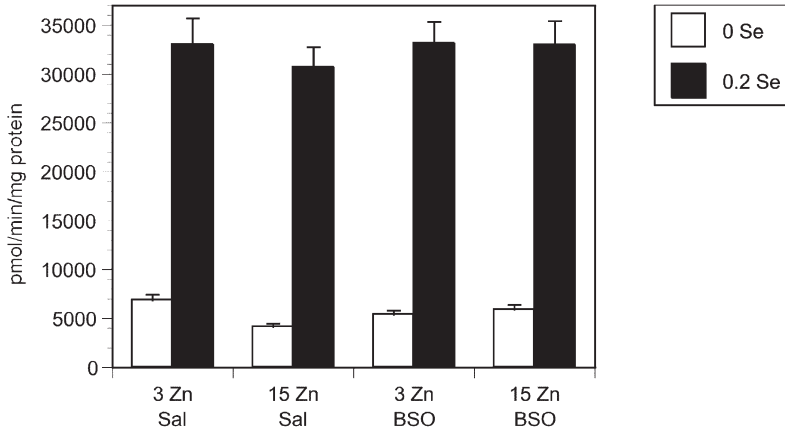

Fig. 3. The effect of dietary Se, Zn, and BSO on liver Msr activity. Values for Se and Zn are in μg/g diet. BSO/saline: Mice were injected (ip) three times per week with 2 mmol L-buthionine sulfoximine/kg body weight or with the 0.9% saline vehicle. Bars represent means ± SD, N=4 per group. Significant effects (as determined by ANOVA): Se, $p<0.0001$; Zn × BSO/saline, $p<0.035$.

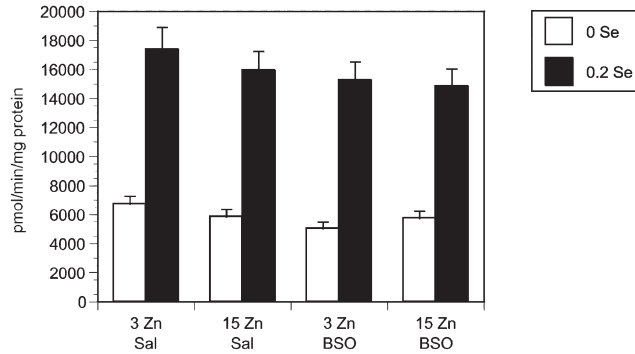

Fig. 4. The effect of dietary Se, Zn, and BSO on kidney Msr activity. Values for Se and Zn are in μg/g diet. BSO/saline: Mice were injected (ip) three times per week with 2 mmol L-buthionine sulfoximine/kg body weight or with the 0.9% saline vehicle. Bars represent means ± SD, N=4 per group. Significant effects (as determined by ANOVA): Se, $p<0.0001$; BSO/saline, $p<0.002$.

Regardless of Se, when Zn was supplemented to the saline-treated mice, liver Msr activity decreased; supplementation of Zn had no effect on liver Msr activity in the BSO-treated mice. Regardless of Se or Zn status, saline treatment (vs BSO) increased kidney Msr activity; this resulted in a significant treatment effect (Fig. 4; $p<0.002$). A three-way interaction (Se × Zn × BSO/saline) affected brain Msr. Compared to Msr activity in Se-deficient mice, Msr activity was increased by Se supplementation in each respective Zn–BSO/saline group except in the mice fed 15 μg Zn/g and

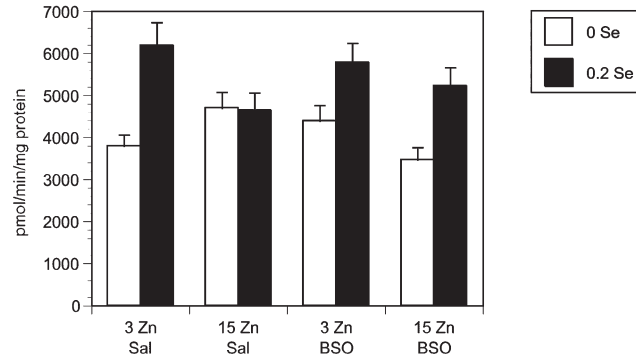

Fig. 5. The effect of dietary Se, Zn, and BSO on brain Msr activity. Values for Se and Zn are in µg/g diet. BSO/saline: Mice were injected (ip) three times per week with 2 mmol L-buthionine sulfoximine/kg body weight or with the 0.9% saline vehicle. Bars represent means ± SD, N=4 per group. Significant effects (as determined by ANOVA): Se, $p<0.0001$; Zn, $p<0.002$; Se χ Zn, $p<0.002$; Se × Zn χ BSO/saline, $p<0.0001$.

injected with saline. In this group, supplementation with Se did not result in an increase in Msr activity.

DISCUSSION

Dietary restriction of Se markedly decreases the activity of Msr from the liver, kidney, and brain. In this experiment, we measured the combined activities of MsrA and MsrB by using a substrate that was a racemic mixture: dabsyl-*R*,*S*-methionine sulfoxide. By using dabsyl-*R*-methionine sulfoxide, Moskovitz and Stadtman *(17)* showed that Se deficiency significantly reduced the activity of MsrB in the liver, kidney, and brain; by using dabsyl-*S*-methionine sulfoxide, they showed that Se deficiency had little, if any, effect on MsrA from the same tissues. Thus, the effects reported in the present experiment are indicative of the effect of Se status on MsrB. Furthermore, increased MetO relative methionine was seen in protein from the liver of Se-deficient mice compared to mice fed the Se-adequate diet (Fig. 2). This would indicate decreased repair by MsrB of oxidized methionine in protein from Se-deficient mice. These data provide further support of the antioxidant function of the selenoprotein MsrB.

The 3-µg Zn/g diet was selected to be low in Zn [the requirement for the mouse is 10 µg/g (11)], but not low enough to cause cyclical feeding and the growth reduction seen in Zn deficiency *(11).* Although food intake was not monitored, the weights of the mice were not affected by dietary Zn (Table 2). The concentration of Zn in the liver (data not shown) was slightly, but not significantly, lower (<5%) in the saline-treated mice fed 3 µg Zn/g compared to saline-treated mice fed 15 µg Zn/g. Thus, the low-Zn

diet was most likely marginal in Zn. Msr activity (combined MsrA and MsrB) in the liver and kidney were not affected by a Zn main effect. However, in the brain, Msr activity somewhat paralleled the response of plasma GPx and TRR activities to dietary treatment. Regardless of BSO/saline treatment, when dietary Se was low, supplementation of Zn had little effect on brain Msr activity. However, when Se was adequate, supplementation of Zn resulted in a decrease in the activity of brain Msr. This resulted in a significant interaction between Se and Zn ($p<0.002$). The consequences and causes of this observation (higher Msr activity in Se-adequate mice fed low Zn compared to Se-adequate mice fed adequate Zn) are yet to be determined. Because MsrB is a Zn-containing enzyme, it was hypothesized that decreasing the dietary Zn would decrease rather than increase the Msr (most likely MsrB) activity. The later unexpected result will have to be further investigated to better characterize the involvement of Zn in MsrB activity. Also, it is unknown to what extent, if any, Zn deficiency will affect the activity of MsrA in various tissues.

Selenium deficiency in rats upregulates hepatic γ-glutamylcysteine synthetase *(22,23),* the same enzyme that is inhibited by BSO. In rats, we have shown that Se deficiency elevates plasma GSH (36% increase) and decreases plasma homocysteine and cysteine *(24).* In the present experiment, we found that Se deficiency in mice decreases plasma homocysteine and cysteine but has no effect on plasma GSH. Thus, it appears that BSO treatment is not confounded by Se deficiency in mice. Treatment with BSO [2 mmol/kg body weight; based on a previous rat study *(25)*] was used to put an additional oxidative stress on the mice. BSO specifically inhibits the synthesis of GSH through inhibition of the enzyme γ-glutamylcysteine synthetase *(26,27).* In rats, when BSO was injected twice per day for the last 7 d of the experiment, blood total GSH decreased 71% *(25).* However, in the present experiment, injection of BSO three times per week for the last 3 wk had no significant effect on plasma GSH (Table 2). In all mice except those fed no supplemental Se and low Zn, BSO treatment slightly, but not significantly, reduced plasma GSH. This, in turn, might explain why BSO had little effect on Msr activity.

The results show that there is an increased concentration of MetO relative to methionine in the liver of mice fed a Se-deficient diet. The increase in MetO concentration in Se-deprived mice was not unexpected, as over half of the known selenoproteins have been found to have antioxidant functions *(28,29).* Also, our results show that marginal Zn deficiency has little effect on Msr, that the oxidative effects of limited BSO treatment did not upregulate Msr activity, and that Se deficiency significantly lowers the activity of liver, kidney, and brain Msr. The design of the experiment, however, did not allow us to draw conclusions on the effect of marginal Se deficiency or supranutritional Se on the activity of Msr. Animal and human studies have shown cancer protection to be associated with intakes of Se greater than those needed for maximal activity of the most studied selenoprotein, GPx. It is not known if the activity of MsrB (or MsrA) is

maximal at the dietary requirement of Se for the mouse [0.15 μg Se/g *(11)*]. Interestingly, TRR appears to be regulated by a different mechanism—one that is dependent on both Se supply as well as oxidative stress level and certain phytochemicals in the diet *(30,31).* This might be important to MsrA and MsrB activities, as these enzymes are linked to the thioredoxin–TRR system to provide reducing equivalents needed for reduction of MetO to methionine. Thus, future studies are planned to determine the effect of supranutritional Se on Msr A and MsrB activities.

REFERENCES

1. J. Gao, D. H. Yin, Y. Yao, et al., Loss of conformational stability in calmodulin upon methionine oxidation, *Biophys. J.* **74,** 1115–1134 (1998).
2. R. Grimaud, B. Ezraty, J. K. Mitchell, et al., Repair of oxidized proteins. Identification of a new methionine sulfoxide reductase, *J. Biol. Chem.* **276,** 48,915–48,920 (2001).
3. E. R. Stadtman, J. Moskovitz, B. S. Berlett, and R. L. Levine, Cyclic oxidation and reduction of protein methionine residues is an important antioxidant mechanism, *Mol. Cell. Biochem.* **234–235,** 3–9 (2002).
4. J. Moskovitz, Methionine sulfoxide reductases: ubiquitous enzymes involved in antioxidant defense, protein regulation, and prevention of aging-associated diseases, *Biochim. Biophys. Acta* **1703,** 213–219 (2005).
5. J. Moskovitz, J. M. Poston, B. S. Berlett, N. J. Nosworthy, R. Szczepanowski, and E. R. Stadtman, Identification and characterization of a putative active site for peptide methionine sulfoxide reductase (MsrA) and its substrate stereospecificity, *J. Biol. Chem.* **275,** 14,167–14,172 (2000).
6. J. Moskovitz, V. K. Singh, J. Requena, B. J. Wilkinson, R. K. Jayaswal, and E. R. Stadtman, Purification and characterization of methionine sulfoxide reductases from mouse and *Staphylococcus aureus* and their substrate stereospecificity, *Biochem. Biophys. Res. Commun.* **290,** 62–65 (2002).
7. G. V. Kryukov, R. A. Kumar, A. Koc, Z. Sun, and V. N. Gladyshev, Selenoprotein R is a zinc-containing stereo-specific methionine sulfoxide reductase, *Proc. Natl. Acad. Sci. USA* **99,** 4245–4250 (2002).
8. S. Bar-Noy and J. Moskovitz, Mouse methionine sulfoxide reductase B: effect of selenocysteine incorporation on its activity and expression of the seleno-containing enzyme in bacterial and mammalian cells, *Biochem. Biophys. Res. Commun.* **297,** 956 (2002).
9. T. C. Squier, Oxidative stress and protein aggregation during biological aging, *Exp. Gerontol.* **36,** 1539–1550 (2001).
10. J. Moskovitz, H. Weissbach, and N. Brot, Cloning the expression of a mammalian gene involved in the reduction of methionine sulfoxide residues in proteins, *Proc. Natl. Acad. Sci. USA* **93,** 2095–2099 (1996).
11. National Research Council, *Nutrient Requirements of Laboratory Animals,* 4th ed., National Academy Press, Washington, DC pp. 80–102 (1995).
12. P. G. Reeves, F. H. Nielsen, and G. C. Fahey, Jr., AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet, *J. Nutr.* **123,** 1939–1951 (1993).
13. P. G. Reeves, Components of the AIN-93 diets as improvements in the AIN-76A diet, *J. Nutr.* **127,** 838S–841S (1997).
14. P. Durand, L. J. Fortin, S. Lussier-Cacan, J. Davignon, and D. Blache, Hyperhomocysteinemia induced by folic acid deficiency and methionine load—applications of a modified HPLC method, *Clin. Chim. Acta* **252,** 83–93 (1996).

15. D. Paglia and W. Valentine, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, *J. Lab. Clin. Med.* **70,** 158–169 (1967).
16. K. J. Hintze, K. A. Wald, H. Zeng, E. H. Jeffery, and J. W. Finley, Thioredoxin reductase in human hepatoma cells is transcriptionally regulated by sulforaphane and other electrophiles via an antioxidant response element, *J. Nutr.* **133,** 2721–2727 (2003).
17. J. Moskovitz and E. R. Stadtman, Selenium-deficient diet enhances protein oxidation and affects methionine sulfoxide reductase (MsrB) protein level in certain mouse tissues, *Proc. Natl. Acad. Sci. USA* **100,** 7486–7490 (2003).
18. J. Moskovitz, B. S. Berlett, J. M. Poston, and E. R. Stadtman, The yeast peptide–methionine sulfoxide reductase functions as an antioxidant in vivo, *Proc. Natl. Acad. Sci. USA* **94,** 9585–9589 (1997).
19. K. L. Maier, A. G. Lenz, I. Beck-Speier, and U. Costabel, Analysis of methionine sulfoxide in proteins, *Methods Enzymol.* **251,** 455–461 (1995).
20. M. R. Schilling, H. P. Khanjian, and L. A. C. Souza, Gas chromatographic analysis of aminoa acids as ethyl chloroformate derivatives, *J. Am. Inst. Conserv.* **35,** 45–59 (1996).
21. D. C. Turnell and J. D. Cooper, Rapid assay for amino acids in serum or urine by precolumn derivatization and reversed-phase liquid chromatography, *Clin. Chem.* **28,** 527–531 (1982).
22. K. E. Hill and R. F. Burk, Effect of selenium deficiency and vitamin E deficiency on glutathione metabolism in isolated rat hepatocytes, *J. Biol. Chem.* **257,** 10,668–10,672 (1982).
23. K. E. Hill and R. F. Burk, Effect of selenium deficiency on the disposition of plasma glutathione, *Arch. Biochem. Biophys.* **240,** 166–171 (1985).
24. E. O. Uthus, K. Yokoi, and C. D. Davis, Selenium deficiency in Fisher-344 rats decreases plasma and tissue homocysteine concentrations and alters plasma homocysteine and cysteine redox status, *J. Nutr.* **132,** 1122–1128 (2002).
25. E. O. Uthus and Y. J. Kang, Effect of buthionine sulfoximine on the response to arsenic deprivation in female rats, *J. Trace Elements Exp. Med.* **11,** 29–36 (1998).
26. O. W. Griffith, Depletion of glutathione by inhibition of biosynthesis, *Methods Enzymol.* **77,** 59–63 (1981).
27. O. W. Griffith, Mechanism of action, metabolism, and toxicity of buthionine sulfoximine and its higher homologs, potent inhibitors of glutathione synthesis, *J. Biol. Chem.* **257,** 13,704–13,712 12 (1982).
28. V. N. Gladyshev and D. L. Hatfield, Selenocysteine-containing proteins in mammals, *J. Biomed. Sci.* **6,** 151–160 (1999).
29. H. Tapiero, D. M. Townsend, and K. D. Tew, The antioxidant role of selenium and seleno-compounds, *Biomed. Pharmacother.* **57,** 134–144 (2003).
30. M. M. Berggren, J. F. Mangin, J. R. Gasdaka, and G. Powis, Effect of selenium on rat thioredoxin reductase activity: increase by supranutritional selenium and decrease by selenium deficiency, *Biochem. Pharmacol.* **57,** 187–193 (1999).
31. J. W. Finley, A. Sigrid-Keck, R. J. Robbins, and K. J. Hintze, Selenium enrichment of broccoli: interactions between selenium and secondary plant compounds, *J. Nutr.* **135,** 1236–1238 (2005).