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Journal of Nutritional Biochemistry 12 (2001) 88–94

THE JOURNAL OF Nutritional Biochemistry

Glutathione peroxidase activity and selenoprotein W levels in different brain regions of selenium-depleted rats

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Received April 24, 2000; accepted August 28, 2000

Abstract

Previous studies in selenium (Se)-depleted sheep and rats showed that selenoprotein W (SeW) levels decreased in all tissues except brain. To further investigate this depletion in different parts of the brain, second generation Se-depleted rats were used. Dams consumed a Se-deficient basal diet during gestation and lactation, and deficient rats were obtained by continuation on the same diet. Control rats were fed a diet with 0.1-mg Se/kg diet after weaning. Glutathione peroxidase (GPX) activities were measured for comparative purposes to SeW levels. GPX activity in muscle, skin, spleen, and testis increased about 4-fold with Se repletion and reached a plateau after 6 or 10 weeks, but GPX activity decreased to almost one tenth of the original activity with continuous Se depletion. In contrast, GPX activities increased, rather than declined, in various brain regions (cortex, cerebellum, and thalamus) with time of feeding the deficient diet. An experiment with first generation rats, however, indicated that GPX activity was significantly lower in these three brain regions from rats fed the deficient diet as compared to rats fed the supplemented diet. SeW levels in skin, spleen, muscle, and testis were undetectable in weanling rats, but became detectable after 6 weeks of Se repletion. In contrast, the expression of SeW in cortex, cerebellum, and thalamus was not significantly affected by Se depletion, but increased SeW levels occurred only in thalamus with Se supplementation. The results with GPX using first and second generation rats suggest that there are "mobile" and "immobile" GPX fractions in the brain.

Keywords: selenoprotein W; glutathione peroxidase; rat brain; Western blot

1. Introduction

Selenium (Se) was suggested to be an essential element for rats in 1957 [1]. Since then, a number of selenoenzymes have been identified. They are the glutathione peroxidase (GPX) family, containing at least four different selenoenzymes [2–5], the iodothyronine deiodinase family, containing types I, II, and III deiodinases [6–9], thioredoxin reductases [10,11], and selenophosphate synthetase [12]. All of these selenoenzymes contain one Se atom per polypeptide chain, although some of the GPXs are multimeric proteins. Selenoprotein P [13,14] and selenoprotein W (SeW) are selenoproteins without a known function. Selenoprotein P, the major Se compound in plasma, contains 10 selenocysteine residues [14]. SeW is a low molecular weight protein originally reported as a missing component in Se-deficient animals suffering from white muscle disease [15]. It was first purified from cytosol of rat skeletal muscle [16]. The cDNA sequence of rat SeW is composed of 672 nucleotides. The 5′ untranslated region consists of 51 bases, followed by a 267-base coding sequence. The presence of one selenocysteine codon, TGA, corresponds to amino acid residue 13 in the predicted sequence and confirms that the protein is indeed a selenoprotein [17,18]. The 3′ untranslated region is required for selenocysteine cotranslation [18,19].

Second and third generation Se-depleted rats have conveniently been used to study Se metabolism. By using this approach, whole brain and reproductive and endocrine organs have been shown to have priority for Se over other tissues studied in third generation depleted rats [20]. In second generation depleted rats, liver Se and GPX activity were reduced by 99%, liver glutathione-S-transferase activity increased by 114%, plasma thyroxine concentration increased by 67%, plasma triiodothyronine decreased by 23%, and the plasma triiodothyronine:thyroxine ratio decreased

Published with the approval of Oregon State University Experiment Station as technical paper no. 11,624.

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0955-2863/01/$ – see front matter
PII: S0955-2863(00)00130-3

by 55%, as compared to controls [21]. In other work from this same laboratory, high retention values were found for phospholipid hydroperoxidase GPX (GPX_4) and cellular GPX (GPX_1) in the cerebrum [22], but this was the only brain fraction investigated, and other fractions need to be studied. Neither of these research groups investigated the linear responses with time.

SeW is present in most tissues from rats fed a Se-adequate diet, but is highest in skeletal muscle, brain, testis, and spleen [23,24]. In Se deficiency, SeW was depleted from all tissues examined in rat [23] and sheep [25], except brain tissue. This raises the question as to whether SeW might be important in brain functions. Other researchers reported that Se distribution and selenoprotein expression are regulated differently among different tissues when Se is limited [26,27]. The organ-specific modulation of SeW expression may relate to the most important sites of function in Se deficiency and influence the response of the Se-deficient animals during Se repletion. Therefore, the present experiment was conducted to further investigate the effects of depletion and repletion of Se on SeW expression in different parts of the brain of second generation Se-depleted rats. GPX activity in different parts of the brain was also determined for comparative purposes. In addition, GPX and SeW were determined in other tissues of rats for comparison to the brain. This was followed by an experiment with first generation rats because the results with GPX in second generation rats were at variance with those of other researchers [28].

2. Materials and methods

2.1. Animals

Second generation Se-depleted rats were used to examine the expression of SeW in different tissues. Although these are not truly second generation rats, they will be referred to as such for convenience. Pregnant Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA USA) were fed a Se-deficient diet during gestation and lactation. Weanling rats (45 male and 45 female) were fed either the Se-deficient diet (4 μg Se/kg) or this diet supplemented with 0.1 mg Se as sodium selenite per kg. The basal diet was composed of 30% torula yeast (Rhinelander Paper Co., Rhinelander, WI USA), 51.5% sucrose, 9% purified cellulose (Solka Floc, Brown Co., Berlin, NH USA), 5% corn oil, 3.5% AIN-93M mineral mix without Se and 1% AIN-76 vitamin mix [29], 0.3% L-methionine, and 0.2% choline citrate. This was followed by an experiment with first generation male rats where weanling rats (5/diet) were fed either the Se-deficient diet or this same diet plus 0.1 mg Se per kg as sodium selenite for 14 weeks. The animals were anesthetized with sodium pentobarbital (80 mg/kg i.p.), and rat tissues (brain, muscle, skin, spleen, and testis) were removed and frozen immediately afterward at −80°C. Rat brains were dissected as cortex, cerebellum, and thalamus before freezing. Tissues from second generation rats (5 animals each group) were collected at weaning, 2, 6, 10, and 14 weeks after weaning. SeW levels and GPX activities were measured on all tissues examined. Because no differences were found due to gender, only the values for the male rats are presented.

The animals were not perfused before the tissues were collected, but this is not considered a problem because SeW is absent in the blood and GPX activity is very low in blood from deficient animals [24,26].

2.2. Western blot analysis

Western blots were determined as described previously [24]. Briefly, tissues were homogenized in Tris buffer [24], the homogenates centrifuged to obtain cytosolic extracts, protein content analyzed [30], tissues extracts electrophoresed [31], proteins transferred onto nitrocellulose membranes [32] hybridized with purified SeW polyclonal antibody [24], membranes washed and incubated with secondary antibody, washed to eliminate excess secondary antibody, blots incubated with ECL chemiluminescent reagent (Amersham Life Science, Inc., Arlington Heights, IL USA), and exposed to Hyperfilm ECL (Amersham, IL USA). Developed films were scanned with a Personal Densitometer SI (Molecular Dynamics, Sunnyvale, CA USA) and analyzed by the ImageQuaNT program (Molecular Dynamics, Sunnyvale, CA USA).

2.3. GPX activities assay

Cellular GPX was measured by a coupled enzyme method using hydrogen peroxide as a substrate [33] (Beckman DU Series 64 spectrophotometer, Beckman Instruments, Inc., Fullerton, CA USA). GPX activity was expressed as nmole nicotinamide adenine dinucleotide phosphate oxidized per min per mg protein.

2.4. Statistical analysis

Data were examined for equal variance and normal distribution prior to statistical analysis. Mean values were compared by analysis of variance to determine whether there were statistically significant differences. If differences were found, the Fisher's least-significant difference method [34] was used to determine which values were significantly different. A significant level of 5% was adopted for all comparisons.

3. Results

3.1. GPX activity in rat tissues

As shown in *Figure 1*, GPX activities in muscle (*A*), spleen (*B*), skin (*C*), and testis (*D*) declined with Se deple-

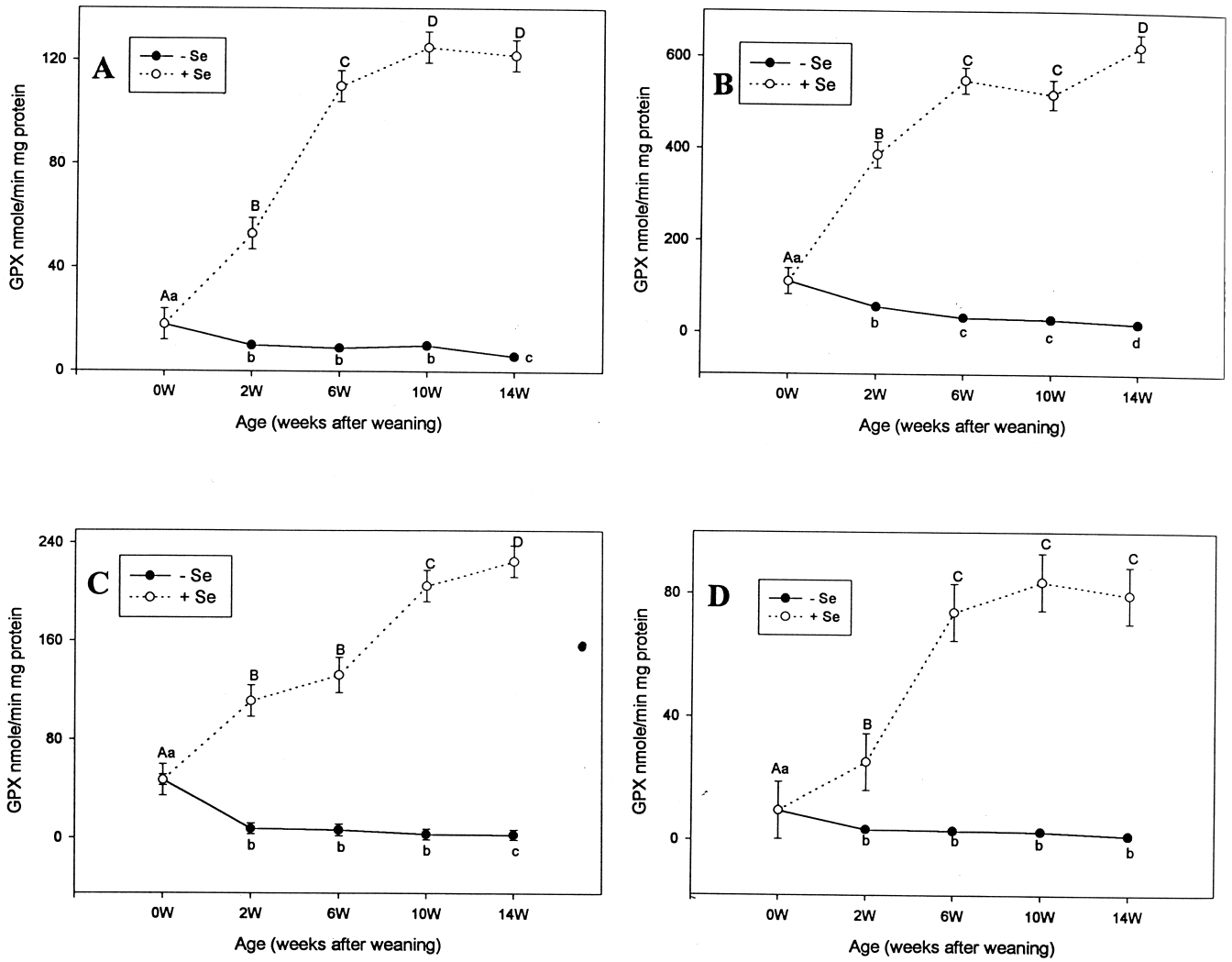

Figure 1 Glutathione peroxidase (GPX) activity in muscle (A), spleen (B), skin (C), and testis (D) from Se-deficient and -supplemented rats. GPX activity is expressed as nmole nicotinamide adenine dinucleotide phosphate oxidized per min per mg protein. Each point represents means of 5 animals ± SEM, and those within a treatment group with different letters are significantly different ($P < 0.05$).

tion. When Se was supplied in the diet after weaning, GPX activities increased about 5-fold in all the tissues. GPX activity in muscle (*Figure 1A*), spleen (*Figure 1B*), and testis (*Figure 1D*) reached a plateau after 6 weeks of Se supplementation, whereas in skin (*Figure 1C*), the activity was still increasing at the end of the experiment.

Surprisingly, the GPX activities were higher at 6 and 10 weeks in the cortex (*Figure 2A*), higher at 6 weeks in cerebellum (*Figure 2B*), and at all times in the thalamus (*Figure 2C*) than initial values. Except initially, GPX activity in cerebellum (*Figure 2B*) was higher from Se-supplemented rats at all times, higher in thalamus at 6 weeks and afterwards (*Figure 2C*), but lower at 2 weeks and higher only at 14 weeks in cortex (*Figure 2A*) when compared to deficient rats. The GPX activity reached a plateau at 10 weeks in cortex (*Figure 2A*) and cerebellum (*Figure 2B*), but it was still increasing at 14 weeks in thalamus (*Figure 2C*) of Se-supplemented rats.

3.2. SeW expression in rat tissues

SeW levels are shown for the three brain regions from Se-supplemented and deficient rats in *Figure 3*. No significant differences were found in SeW levels in cortex (*Figure 3A*) and cerebellum (*Figure 3B*) between Se-depleted and -repleted rats, even after 14 weeks. In contrast, the SeW levels in the thalamus (*Figure 3C*) were significantly higher ($P < 0.05$) in repleted than depleted rats both at 6 and 14 weeks.

SeW was undetectable by the method used in muscle, skin, spleen, and testis in Se-depleted rats (initial values), but was detectable after 6 weeks of Se supplementation (*Figure 4*). However, after 6 weeks of Se supplementation, SeW was detected in muscle (*Figure 4A*), spleen (*Figure 4B*), skin (*Figure 4C*), and testis (*Figure 4D*), and the values were significantly higher ($P < 0.05$) at 14 weeks than at 6 weeks in muscle, spleen, and skin. In contrast, there were no

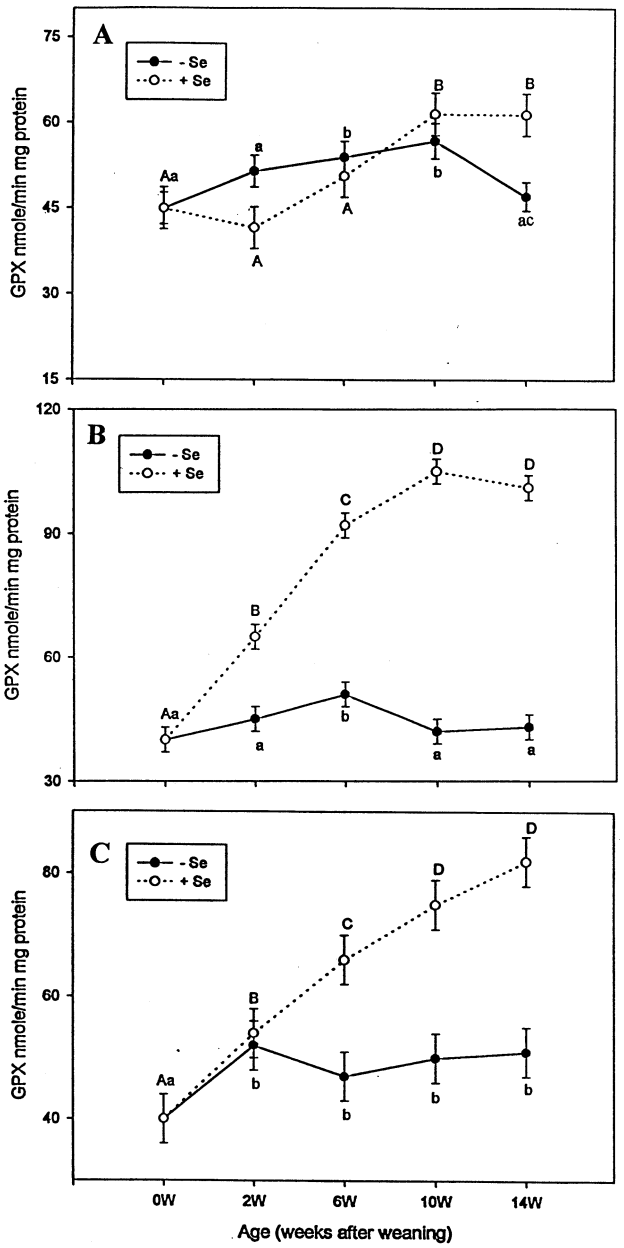

Figure 2 Glutathione peroxidase (GPX) activity in cortex (A), cerebellum (B), and thalamus (C) from Se-deficient and -supplemented rats. GPX activity is expressed as nmole nicotinamide adenine dinucleotide phosphate oxidized per min per mg protein. Each point represents means of 5 animals ± SEM. Points within a treatment group with different letters are significantly different ($P < 0.05$). The values of the cerebellum from the Se-supplemented rats are significantly higher than the values from deficient rats at 2 weeks, but significantly lower at 14 weeks. The values for thalamus from deficient rats are significantly lower than values from Se-supplemented rats at 6 weeks and afterwards.

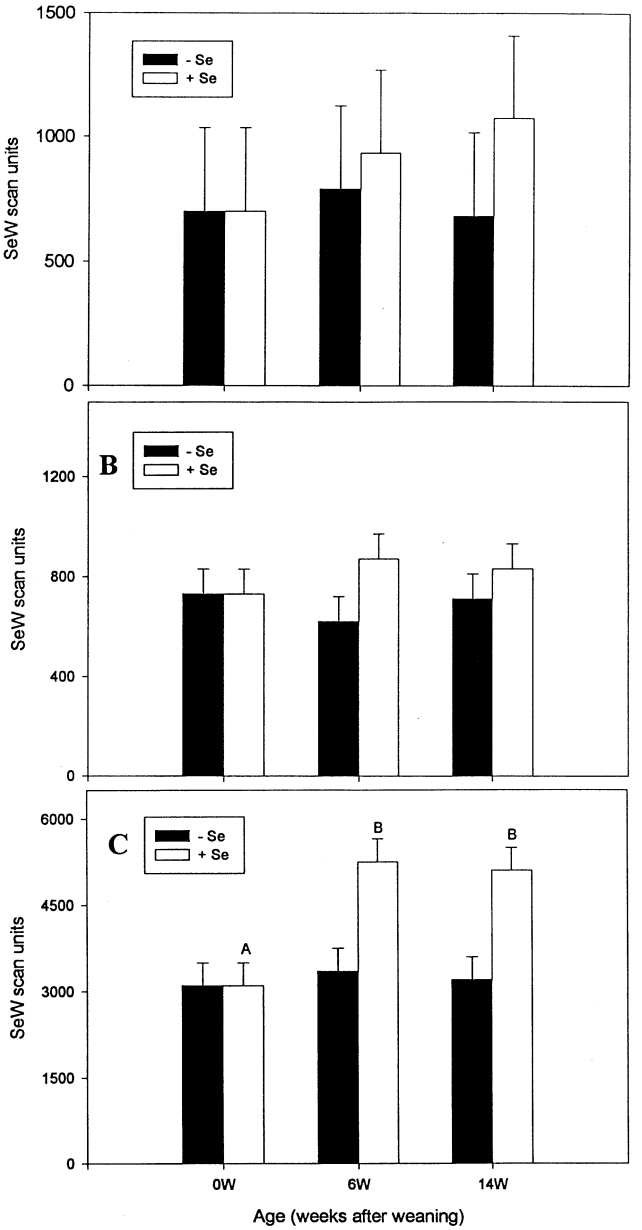

Figure 3 SeW expression in cortex (A), cerebellum (B), and thalamus (C) from Se-deficient and -supplemented rats. SeW levels are expressed as scan units. The bars represent means of 5 animals ± SEM, and those with different letters are significantly different ($P < 0.05$).

differences in the SeW content in testis between 6 and 14 weeks.

Se deficiency resulted in significantly lower GPX activity in muscle, liver, cortex, cerebellum, and thalamus as compared to Se-supplemented first generation rats (*Table 1*). Even though SeW was not detected in muscle from Se-deficient rats, there were no differences in this selenoprotein in cortex, cerebellum, or thalamus between deficient and supplemented rats (data not shown).

3. Discussion

Rather surprising, the GPX activity increased in the three brain regions of Se-deficient second generation rats (*Figure 2*). Following parturition, similar patterns were found for women with low Se status, where curvilinear responses were obtained for GPX activity and Se levels in plasma and milk [35]. Other studies indicated that there was no differ-

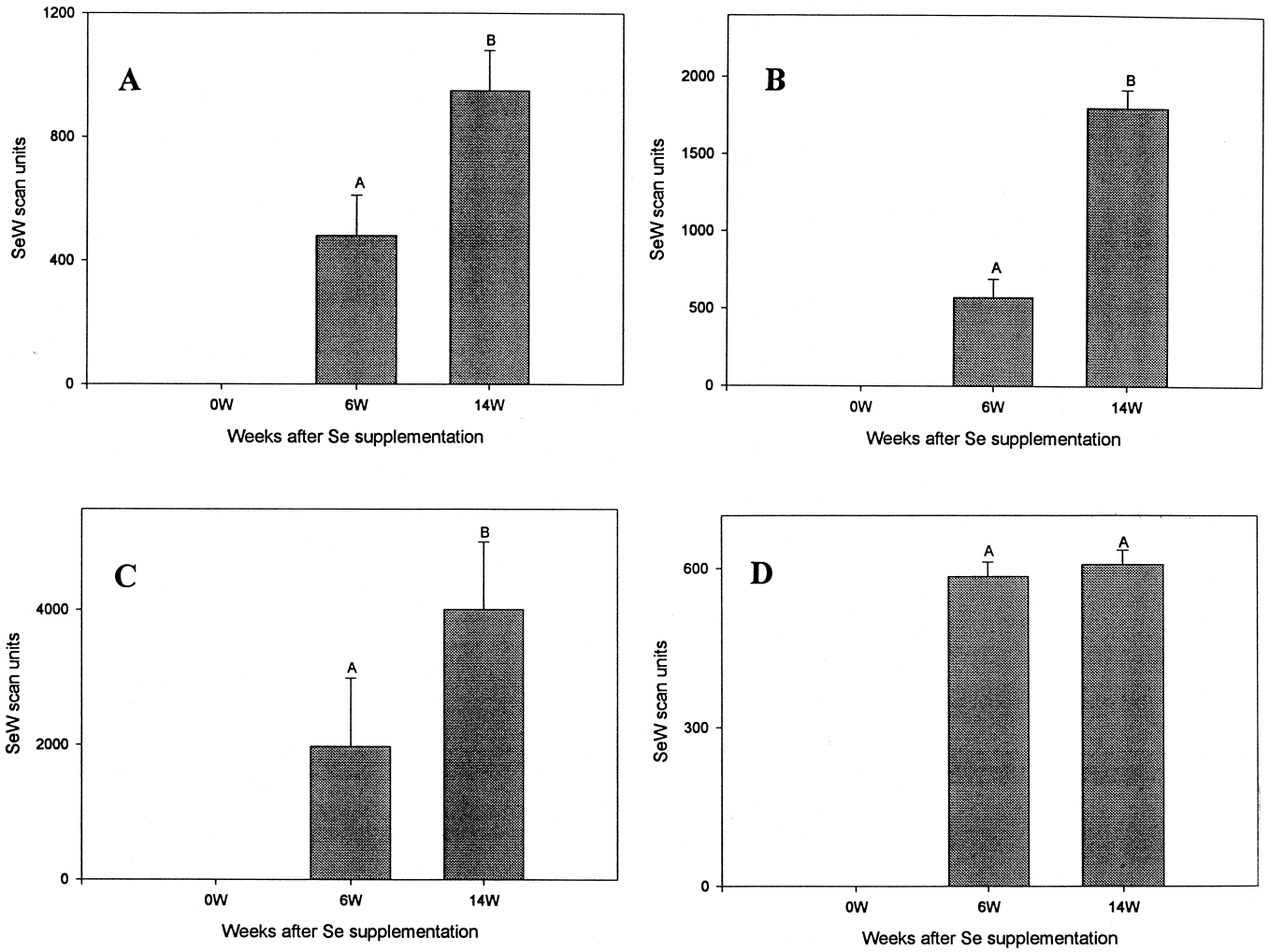

Figure 4 SeW levels in muscle (A), spleen (B), skin (C), and testis (D) after Se supplementation. SeW levels are expressed as scan units and represent means of 5 animals ± SEM, and those with different letters are significantly different ($P < 0.05$).

ence of GPX in colostrum from women of low Se status versus those with adequate Se intake [36]. Apparently, GPX is very critical to the brain and in colostrum, and Se is transferred from less critical tissues to meet the requirements of critical tissues. This has been referred to as biological readjustment, and this process may even result in temporary peroxidation [35]. This is consistent with data by other researchers who presented evidence for the redistribution of Se in the organism during Se deficiency [37]. In addition to the brain, endocrine and male reproductive organs have been reported to retain Se at the expense of other tissues such as liver, kidney, and muscle when supplies of this element are limited [20].

Table 1
Glutathione peroxidase in muscle, liver, cortex, cerebellum, and thalamus from first generation rats fed either a selenium (Se)-deficient or a supplemented diet

Tissues	Se-Deficient	Se-Supplemented
Muscle	36 ± 5*	73 ± 13
Liver	60 ± 7*	751 ± 136
Cortex	53 ± 2*	63 ± 2
Cerebellum	65 ± 4*	93 ± 3
Thalamus	105 ± 17*	151 ± 4

Glutathione peroxidase activity is expressed as nmole of nicotinamide adenine dinucleotide phosphate oxidized per min per mg protein.

* Significantly lower than that from Se-supplemented rats.

The results with GPX in the brain regions of second generation Se-deficient rats were different (*Figure 2*) than reported by another research group for the whole brain [28]. For this reason, the experiment with first generation rats was conducted (*Table 1*), which resulted in similar data to these researchers. Because GPX activity decreased in first generation rats (*Table 1*) but not in second generation rats (*Figure 2*), it is proposed that there are "mobile" GPX and "immobile" GPX in the brain. This concept is consistent with the data obtained by other researchers; the retention factor was calculated for various tissues, and priority of Se is to the brain, and reproductive and endocrine organs [20,22,37]. All of the "mobile GPX" is postulated to be depleted in the

second generation rats, and this is the reason additional depletion did not occur. Consequently, further depletion of GPX did not occur because only the "immobile" fraction was left, and the reason for increases in activity of this selenoenzyme was because of biological readjustment to meet critical needs in the brain.

GPX activity decreased in muscle, spleen, skin, and testis during Se deficiency (*Figure 1*). This is consistent with prior studies [23,37] with Se deficiency and, thus, most of the GPX in these organs appears to be "mobile". Others have referred to GPX in these organs as "expendable Se pools" [20,38]. SeW in these tissues were undetectable in muscle, spleen, and testis in Se-deficient rats. The more rapid response of SeW to Se supplementation in testis (*Figure 4D*) compared to muscle (*Figure 4A*) or spleen (*Figure 4B*) is consistent with our earlier work [39].

Even though no decreases in GPX activity occurred in cortex, cerebellum, and thalamus with Se deficiency (*Figure 2*), Se supplementation resulted in increases of GPX activity of 40%, 100%, and 60% in the cortex, cerebellum, and thalamus, respectively. Cerebellum had the highest GPX activity among the three parts of the brain. This data is consistent with that of others who showed that of the tissues examined, the retention for GPXs (both GPX_1 and GPX_4) was among the highest for cerebellum [22].

The response of SeW to Se supplementation in the three regions of the brain further emphasizes the importance of studying specific regions of this organ rather than composite samples of the whole brain (*Figure 3*). It is obvious that the regulations of selenoprotein synthesis by various parts of the brain are different, and this organ apparently regulates its selenoprotein levels to compensate for Se deficiency. These results provide evidence for the existence of distinct mechanisms for control of expression of different selenoproteins in the brain [20,22,28]. The fact that GPX and Se decreased in the brain of deficient animals, whereas SeW [25] and thioredoxin reductase [28] remained constant suggests that they are more critical to this organ than some other selenoproteins such as "mobile" GPX. This is consistent with data by another research group who indicated that the supply to other selenoproteins has priority over that for GPX_1 Se as selenoprotein P but not as GPX was taken up in significantly higher amounts by the brain from Se-deficient compared to Se-supplemented rats [40], suggesting a critical function of this selenoprotein in this organ. SeW is a selenoprotein with unknown functions, but glutathione was demonstrated to be bound to it through a disulfide bond [41]. Thus, it would be reasonable to conclude that SeW may possess some antioxidant properties that would be consistent with functions of some other selenoproteins [42].

Previously, gender differences were found in SeW levels in skin from rat [43], but no differences were found in the present study (data not shown). The reason for this disagreement is not known, but could be due to at least two factors. Second generation Se-depleted rats were used in the present study in contrast to first generation rats in the earlier study. In the prior study, rats were fed commercial chow and had not been depleted of Se, whereas the rats in the present investigation were depleted and fed a purified diet.

Acknowledgments

This research was supported, in part, by Public Health Service Research Grant number DK 38341 from the National Institute of Diabetes and Digestive and Kidney Diseases.

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