The Necessity of Dietary Vitamin B₆ to Selenium Biopotency for Tissue Selenium and Glutathione Peroxidase in Rats

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Summary Necessity of dietary vitamin B₆ to the biopotency of selenium (Se) for the levels of Se and glutathione peroxidase (GSH-Px) in tissues was investigated. Male Wistar 12-week-old rats were fed a vitamin B₆-Se-deficient basal diet for 3 week, and then the rats were divided into 6 groups. One group was fed the basal diet, the others were fed the diet supplemented with 250 µg vitamin B₆/100 g as pyridoxine·HCl, or 0.25 mg Se/kg as Na₂SeO₃ (SeL) or DL-selenomethionine (Se-Met), or both (SeL+B₆ or Se-Met+B₆) for 10 week. The levels of Se and GSH-Px in erythrocytes and muscle were significantly higher in vitamin B₆-supplemented groups than in vitamin B₆-deficient groups. There was little effect of this vitamin deficiency on Se level in liver of rats fed SeL; however, a higher Se level in liver was observed in vitamin B₆-deficient rats fed Se-Met than in the corresponding B₆-supplemented rats. A significant decrease of GSH-Px activity in liver was found in vitamin B₆-deficient animals fed Se-Met compared with vitamin B₆-supplemented animals, whereas no significant decrease was observed in those fed SeL. These results suggest that this vitamin is involved in the transport and deliverance of Se in plasma to the other tissues and the incorporation of Se from Se-Met to GSH-Px in liver.

Key Words selenium, selenite, DL-selenomethionine, rats, glutathione peroxidase, vitamin B₆

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Selenium (Se) requirements in animals and humans have been reported following the establishment of its essentiality (1-3); however, the requirements are influenced by other nutrient status. The metabolism of selenomethionine (Se-Met) is believed to follow methionine (Met) biochemical pathways (4). Se-Met is reported to be the main form of Se in natural food (5, 6). The main functioning form of Se is believed to be an integral part of glutathione peroxidase (GSH-Px, EC 1.11.1.9) (7). Since vitamin B₆ is essential to the metabolism of Met, it can be postulated that this vitamin is involved in the metabolic process of Se-Met to supply Se for GSH-Px. An early study reported that vitamin B₆ deficiency decreased the biopotency of Se-Met for GSH-Px in liver of rats, whereas it had equivalent effect on liver GSH-Px activity in vitamin B₆-deficient or -adequate rats fed SeL (8). However, the effect could not be confirmed from the later experiment (9). It should be pointed out that the tissue Se levels were not reported in either of both studies. Recently, Beilstein and Whanger (10) found that the levels of Se and GSH-Px in erythrocytes were lower in vitamin B₆-deficient rats, tissue retention of ⁷⁵Se provided as Se-Met was increased in vitamin B₆-deficient animals, but B₆ status had no significant effect on muscle Se content. The present study was designed to investigate the necessity of dietary vitamin B₆ to the biopotency of Se, Na₂SeO₃ and Se-Met, for the levels of Se and GSH-Px in the tissues of adult rats.

MATERIALS AND METHODS

Animals and diets. Forty-two male 12-week-old Wistar rats purchased from Clea Japan (Tokyo) were housed individually in suspended mesh, stainless steel cages with a 12-h light/dark cycle in a room at 20-22°C. Rats were weighed weekly. Food and deionized water were provided *ad libitum*. The basal diet, formulated by the AIN⁻⁷⁶ purified diet (11), was a 20% vitamin-free casein (Oriental Yeast, Tokyo) diet. Pyridoxine·HCl in vitamin mixture, Na₂SeO₃ in mineral mixture and 0.3% Met were omitted. Fluorometric analysis (12) showed that this diet contained 0.03 mg Se/kg.

Experimental design. Rats weighing 370 ± 13 g (means ± SD) were fed the basal diet for 3 weeks, and then the rats were randomized by weight into six groups (seven rats per group). One group was fed the basal diet (Basal, -B₆), the others were fed the diet supplemented with 250 µg vitamin B₆/100 g as pyridoxine·HCl (Wako Pure Chemical Industries, Osaka) (Basal, +B₆), or 0.25 mg Se/kg as Na₂SeO₃ (Wako Pure Chemical Industries, Osaka) (SeL, -B₆) or DL-selenomethionine (Sigma Chemical) (Se-Met, -B₆), or 0.25 mg Se/kg and 250 µg vitamin B₆/100 g (SeL, +B₆ or Se-Met, +B₆). Each group of rats was fed its respective diet for 10 weeks. The overnight fasted rats were anesthetized with ether and blood was drawn from the abdominal aorta using a heparinized syringe. The plasma was separated from the erythrocytes by centrifugation at room temperature, and then the erythrocytes were washed three times with 0.9% NaCl. Liver and muscle (from left hind leg) were removed. Samples for enzyme assays were stored at -80°C, and

the other samples were stored at -30°C until analysis. Approximately 0.5 g of muscle or liver was homogenized with an ultra high speed homogenizer in five volumes of 0.25 M sucrose containing 0.25 mM EDTA in 0.1 M potassium phosphate buffer, pH 6.8. The homogenate was centrifuged ($40,000 \times g$, 20 min at 4°C , model 20 PR-52D, RPR 20 rotor, Hitachi), and the supernatant was used for enzyme and protein assays.

Se in diets and tissues was determined with fluorometric method (12). Enzymes were assayed at 25°C unless otherwise indicated. GSH-Px in tissues was assayed by the coupled enzyme method (13) using H_2O_2 as the substrate (14). Plasma aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) were assayed using prepared enzymatic kits (Wako Pure Chemical Industries, Osaka, Code 275-34101 and 271-34201, respectively). Protein was determined by the method of Lowry *et al.* (15). Hemoglobin in erythrocytes was determined from absorption at 540 nm using prepared kit (Wako Pure Chemical Industries, Osaka, Code 279-10701). Enzyme activities were expressed as per mg protein, but it was expressed per mg hemoglobin in erythrocytes. Liver cystathionase (EC 4.4.1.1) activity was assayed by the Matsuo and Greenberg method (16); a unit of cystathionase activity is defined as the amount that yields 1 μmol of α -ketobutyric acid formed per min for cystathionase.

Statistical analysis. The data were given as means $\pm$ SEM and performed using analysis of variance and tested using the Student-Newman-Keuls procedure (17).

RESULTS AND DISCUSSION

Final body weight, liver weight, and vitamin B₆-deficient enzymes

Table 1 shows that the rats fed vitamin B₆-deficient diets gained less significantly final body weight and liver weight than the rats fed vitamin B₆-supplemented diets, regardless of dietary levels or chemical forms of Se. There were no significant differences among vitamin B₆-supplemented or deficient groups. Further, the activities of vitamin B₆-dependent enzymes, plasma aspartate aminotransferase, alanine aminotransferase, and liver cystathionase, were significantly lower in vitamin B₆-deficient groups than in vitamin B₆-supplemented groups (Table 2), regardless of the addition of Se, which suggested that vitamin B₆ deficiency was produced in the animals fed these vitamin B₆-deficient diets. The differences observed from our study can be attributed to vitamin B₆ deficiency.

Se levels in plasma, erythrocytes, muscle and liver

Se-Met caused a large amount of Se to be retained in erythrocytes, muscle, and liver compared with SeL (Tables 3 and 4). The higher tissue Se contents in Se-Met groups compared with SeL groups were assumed to be that Se-Met incorporated in the general protein substituted Met (4). Even though SeL was somewhat less well absorbed than Se-Met, intestinal absorption of both chemical forms was greater

Table 1. Effect of vitamin B₆ on growth and liver weight in rats.

Treatment	Final weight		Liver weight	
	-B ₆	+B ₆	-B ₆	+B ₆
			(g)	
Basal	377±9 ^{ax}	473±9 ^{ay}	9.0±0.3 ^{ax}	11.8±0.2 ^{ay}
SeL	380±3 ^{ax}	488±12 ^{ay}	9.1±0.2 ^{ax}	11.5±0.4 ^{ay}
Se-Met	380±8 ^{ax}	470±7 ^{ay}	9.2±0.3 ^{ax}	11.7±0.3 ^{ay}

Rats fed a vitamin B₆-Se-deficient diet for 3 weeks were then either depleted further (Basal), or substituted with the diet supplemented with 250 µg vitamin B₆/100 g as pyridoxine·HCl (Basal+B₆), or 0.25 mg Se/kg as Na₂SeO₃ (SeL), or DL-selenomethionine (Se-Met), or Se and vitamin B₆ (SeL+B₆ or Se-Met+B₆) for 10 weeks. The results are means±SEM of seven rats per group. Means in the same column with different superscripts a-c are significantly different as a result of chemical forms and dietary levels of Se at the $p < 0.05$ level. Means within a horizontal row with different superscripts x, y are significantly different as a result of vitamin B₆ status at the $p < 0.05$ level.

Table 2. Plasma aminotransferase and hepatic cystathionase activities.¹

Treatment	PALT ²		PAST ³		Cystathionase	
	-B ₆	+B ₆	-B ₆	+B ₆	-B ₆	+B ₆
	IU/mg protein				U/mg protein ⁴	
Basal	3±1 ^{ax}	11±1 ^{ay}	33±4 ^{ax}	77±13 ^{ay}	10±3 ^{ax}	51±3 ^{ay}
SeL	3±0 ^{ax}	11±1 ^{ay}	31±4 ^{ax}	66±8 ^{ay}	10±2 ^{ax}	50±2 ^{ay}
Se-Met	3±1 ^{ax}	12±1 ^{ay}	33±7 ^{ax}	65±7 ^{ay}	11±1 ^{ax}	50±6 ^{ay}

¹ See Table 1 for explanation of each treatment. ² Plasma alanine aminotransferase.

³ Plasma aspartate aminotransferase. ⁴ A unit of cystathionase activity is defined as the amount that yields 1 µmol of α-ketobutyric acid formed per minute for cystathionase.

than 90% (18) so that the absorption of two forms of Se, SeL and Se-Met, did not appear to have a critical influence on tissue Se levels; it was attributed to the finding that a large amount of Se absorbed from SeL was excreted *via* urine (18) because SeL-exchangeable components of body Se had higher turnover than Se-Met-containing components (19).

Table 3 shows that vitamin B₆ status had no significant effect on plasma Se concentration. Beilstein and Whanger (10) showed that the retention of ⁷⁵Se was higher in plasma of vitamin B₆-deficient rats compared to supplemented rats. Therefore, Se level in plasma did not have a bearing on intracellular concentration of Se (20), thus the biological effect of Se would not be related to the level of Se in plasma.

Tables 3 and 4 showed that Se levels in erythrocytes and muscle were significantly higher in vitamin B₆-supplemented rats than in vitamin B₆-deficient

Table 3. Effect of dietary vitamin B₆ deficiency on selenium levels in plasma and erythrocytes of rats.¹

Treatment	Plasma		Erythrocytes	
	– B ₆	+ B ₆	– B ₆	+ B ₆
	$\mu\text{g Se/mg protein}$		$\mu\text{g Se/mg hemoglobin}$	
Basal	$5.0 \pm 0.0^{\text{ax}}$	$4.6 \pm 0.2^{\text{ax}}$	$0.79 \pm 0.06^{\text{ax}}$	$1.55 \pm 0.09^{\text{ay}}$
SeL	$6.1 \pm 0.3^{\text{bx}}$	$6.2 \pm 0.2^{\text{bx}}$	$1.08 \pm 0.05^{\text{bx}}$	$2.36 \pm 0.06^{\text{by}}$
Se-Met	$6.2 \pm 0.2^{\text{bx}}$	$6.2 \pm 0.2^{\text{bx}}$	$1.66 \pm 0.14^{\text{cx}}$	$4.14 \pm 0.10^{\text{cy}}$

¹ See Table 1 for explanation of each treatment.Table 4. Effect of dietary vitamin B₆ deficiency on selenium levels in muscle and liver of rats.¹

Treatment	Muscle		Liver	
	– B ₆	+ B ₆	– B ₆	+ B ₆
	$\mu\text{g/g}$			
Basal	$0.08 \pm 0.00^{\text{ax}}$	$0.13 \pm 0.01^{\text{ay}}$	$0.64 \pm 0.04^{\text{ax}}$	$0.50 \pm 0.02^{\text{ay}}$
SeL	$0.09 \pm 0.01^{\text{bx}}$	$0.16 \pm 0.01^{\text{by}}$	$1.00 \pm 0.04^{\text{bx}}$	$0.99 \pm 0.05^{\text{bx}}$
Se-Met	$0.23 \pm 0.01^{\text{cx}}$	$0.44 \pm 0.02^{\text{cy}}$	$1.29 \pm 0.03^{\text{cx}}$	$1.15 \pm 0.05^{\text{cy}}$

¹ See Table 1 for explanation of each treatment.

rats. Beilstein and Whanger (10) found a similar reduction in erythrocyte Se level in vitamin B₆-deficient rats for all forms of Se (SeL, selenocystine and Se-Met); however, vitamin B₆ status had no significant effect on muscle Se level. In view of the lowest degree of exchange of Se in muscle (19), feeding period in this study seems too short to obtain meaningful result. The present data suggest that this vitamin might be involved in the transport or utilization of Se to these tissues from plasma, or in the conjugation or release of Se in plasma protein.

From the data presented in Table 4, Se contents in liver were little affected by vitamin B₆ status when the rats were fed SeL; on the contrary, the vitamin B₆-deficient rats fed Se-Met had higher Se contents than the vitamin B₆-supplemented rats fed Se-Met. This is consistent with a previous report (10) in which whole liver Se content was higher in the vitamin B₆-deficient rats fed SeL and Se-Met compared with that in the corresponding vitamin B₆-supplemented rats. These results indicate that the assimilation of Se by liver might be not affected, but the metabolic conversion of Se-Met to active forms for tissue utilization would be influenced by vitamin B₆ deficiency.

GSH-Px activities in plasma, erythrocytes, muscle and liver

In vitamin B₆-supplemented rats, the addition of Se either from SeL or Se-Met to the diets significantly increased GSH-Px activities in plasma, erythrocytes, muscle, and liver compared with the Basal + B₆ rats (Tables 5 and 6). However, no

Table 5. Effect of dietary vitamin B₆ deficiency on activity of glutathione peroxidase in plasma and erythrocytes of rats.^{1,2}

Treatment	Plasma		Erythrocytes	
	-B ₆	+B ₆	-B ₆	+B ₆
	nmol NADPH/min·mg·prot.		nmol NADPH/min·mg·Hb	
Basal	56±2 ^{ax}	53±4 ^{ax}	220±22 ^{ax}	430±11 ^{ay}
SeL	74±3 ^{bx}	71±3 ^{bx}	298±10 ^{bx}	619±10 ^{by}
Se-Met	66±2 ^{cx}	62±3 ^{cx}	294±33 ^{bx}	607±20 ^{by}

¹See Table 1 for explanation of each treatment. ²The activity is expressed as nmol NADPH oxidized/min/mg protein (plasma) or mg hemoglobin (Hb) (erythrocytes) with H₂O₂ as substrate.

Table 6. Effect of dietary vitamin B₆ deficiency on activity of glutathione peroxidase in muscle and liver of rats.^{1,2}

Treatment	Muscle		Liver	
	-B ₆	+B ₆	-B ₆	+B ₆
	nmol NADPH/min·mg·prot.			
Basal	26±3 ^{ax}	53±9 ^{ay}	344±11 ^{ax}	337±28 ^{ax}
SeL	36±8 ^{ax}	78±8 ^{by}	843±34 ^{bx}	867±28 ^{bx}
Se-Met	28±3 ^{ax}	80±11 ^{by}	731±23 ^{cx}	846±25 ^{by}

¹See Table 1 for explanation of each treatment. ²The activity is expressed as nmol NADPH oxidized/min/mg protein with H₂O₂ as substrate.

significant difference in muscle GSH-Px activity was observed in SeL and Se-Met groups fed vitamin B₆-deficient diets compared with Basal-B₆ group. Vitamin B₆ had little effect on GSH-Px activity in plasma. In contrast, GSH-Px activities in erythrocytes and muscle were significantly higher in vitamin B₆-supplemented groups than in corresponding vitamin B₆-deficient groups, which was well correlated with the magnitude of difference in Se levels of erythrocytes and muscle. From the present data, we found that the biopotency of Se for GSH-Px activities in erythrocytes and muscle was dependent on dietary vitamin B₆ status. The good consistencies of erythrocyte and muscle GSH-Px activities with Se concentrations suggest that the decrease in the levels of Se and GSH-Px was associated with that the transportation and/or the deliverance of Se in plasma to these tissues were affected when the animals were in vitamin B₆-deficiency. This is not consistent with the results obtained by the other investigators using rats (8,10), in which no significant effect was found in erythrocytes by SeL (8) and no significant difference was shown in GSH-Px activity in muscle of rats fed vitamin B₆-deficient vs. supplemented groups (10).

There was no significant difference in GSH-Px activity in liver of vitamin B₆-deficient rats fed SeL compared to vitamin B₆-supplemented rats, whereas a

significant decrease was observed in vitamin B₆-deficient group fed Se-Met compared with vitamin B₆-supplemented group fed Se-Met, which was consistent with that of Se level in this tissue. Our findings are in agreement with the results on rats obtained by the other researcher (8), which can be explained from vitamin B₆-dependent cystathionase and aspartate aminotransferase deficiency (Table 2). The present data suggest that the metabolism of Se-Met is *via* the vitamin B₆-dependent pathway. These implies that when the animals are poor in vitamin B₆ status, the incorporation of Se from Se-Met to GSH-Px is depressed and the retention of Se in liver may occur.

Considering the dependence of selenoprotein P and GSH-Px upon Se intake (21) and the possibility that vitamin B₆ is involved in the active transport of Se in tissues, deficiency of dietary vitamin B₆ would result in the decrease in an available Se even if the diet was normal in Se contents, especially when Se intake was in marginal level or in low Se areas such as the endemic areas of Keshan and Kashin-Beck diseases in China. Further experiments are under study.

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