Effects of Dietary Vitamin B_6 on Selenium Concentrations and Glutathione Peroxidase Activities in Tissues of Rats Fed Sodium Selenite or Seleno-DL-methionine

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ABSTRACT

The effects of vitamin B_6 (B_6) deficiency on selenium (Se) concentrations and glutathione peroxidase (GSH-Px) activities in tissues of rats fed sodium selenite and selenomethionine were studied. Weanling male Wistar rats were fed a B_6-Se deficient diet for 2 weeks, then the rats were divided into 6 groups, one was depleted further, and the others were either fed the diet supplemented with 2.5mg pyridoxine・HCl/kg, or 0.5mg Se/kg as sodium selenite or seleno-DL-methionine (Se-Met), or Se and B_6 for 4 weeks. B_6-deficient rats all gained significantly less weight than their B_6-adequate counterparts. Regardless of chemical forms of Se, the addition of B_6 to the diets significantly increased Se concentration and GSH-Px activity in erythrocytes compared with those of animals fed B_6-deficient diets, Se concentration and GSH-Px activity in plasma were higher in B_6-deficient rats than in B_6-adequate rats. Se retention in muscle, heart, spleen and liver was greater in rats fed Se-Met than in those fed sodium selenite, and B_6 deficiency further intensified this trend. Activity of GSH-Px in muscle, heart, spleen and liver was lower in B_6-deficient rats than their B_6-adequate counterparts, especially in rats fed Se-Met.

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INTRODUCTION

The bioavailability of different feedstuffs as a sources of selenium (Se) for animals has been reported to depend on the chemical forms in which the element is present[1,2], as well as on its concentration. Early studies in animals and human beings suggested that selenomethionine (Se-Met) is more efficiently absorbed and retained than selenite[3,4], however, the incorporation of Se from Se-Met into glutathione peroxidase (GSH-Px) needed the presence of vitamin B_6 (B_6) because Se-Met is an excellent analog for methionine in biochemical reactions[5,6]. Since the main functioning form of Se is believed to be an integral part of GSH-Px [7], Se intakes in humans are mainly from foods and the major form of Se in foods of plant origin is thought to be in the form of Se-Met[8], this study was designed to determine the effect of dietary B_6 on Se concentrations and GSH-Px activities in the tissues of rats fed sodium selenite or seleno-DL-methionine.

MATERIAL AND METHOD

Animals and diets. Weanling male Wistar rats (mean body weight 80.0g) were fed a B_6-Se deficient purified casein-based diet for 2 weeks, which diet was similar in formulation to the AIN^{-76} diet except that 0.3% methionine, pyridoxine and selenite were omitted. After 2 week depletion, the rats were divided into 6 groups, one group was depleted further (Basal), and the other five groups were either fed the diet supplemented with 2.5mg pyridoxine・HCl/kg (Basal+B_6), or 0.5mg Se/kg as sodium selenite (SeL) or seleno-DL-methionine (Se-Met), or Se and B_6 (SeL+B_6 or Se-Met+B_6). Each group was fed its respective diet for 4 weeks.

Sample preparation and analysis. The overnight-fasted rats were anesthetized with ether, blood was drawn from the punctured abdominal aorta using a heparinized syringe. Plasma was separated from erythrocytes by centrifugation, and then the erythrocytes were washed with 0.85% NaCl three times. Muscle (from left hind leg), heart, spleen and liver were removed for the assays of Se concentration and GSH-Px activity. Approximately 0.5 g of muscle or organ samples was homogenized with ultra high speed homogenizer in 5 volumes of cold 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 ×g, 20 min at 4℃) and the supernatant was assayed for GSH-Px activity and protein content. GSH-Px activity in tissues was assayed by the coupled enzyme method[9] using H_2O_2 as the substrate so that only Se-dependent GSH-Px was assayed[10]. Protein concentration in tissues was determined by the method of Lowry et al[11]. The concentration of Se in tissues was determined with modified fluorometric method[12].

RESULTS AND DISCUSSION

There were no significant differences in weight gain in B_6-adequate animals, however, B_6-deficient rats all gained significant less weight than B_6-adequate rats, regardless of chemical form and levels of Se (Fig. 1).

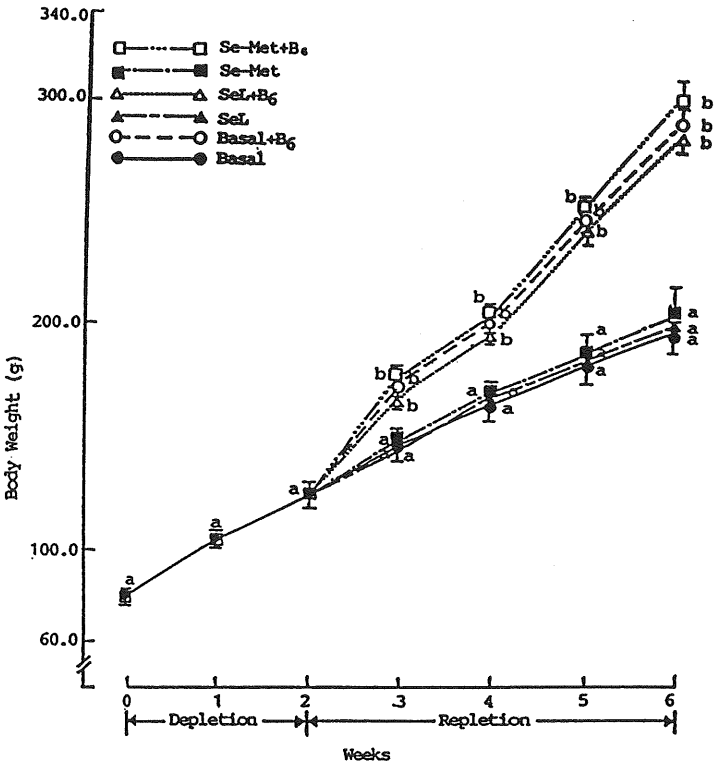

Fig. 1. The mean body weights of rats during vitamin B_6(B_6)-Se depletion and repletion. Weanling male rats fed a B_6-Se deficient diet for 2 wks were then either depleted further (Basal, ●), or repleted with the diet supplemented with 2.5mg pyridoxine・HCl/kg (Basal+B_6, ○), or 0.5mg Se/kg as sodium selenite (SeL, ▲) or seleno-DL-methionine (Se-Met, ■), or Se and B_6 (SeL+B_6, △ ; Se-Met+B_6, □) for 4 wks. Each point represents the means±SEM of five rats per group. Points at any given action week with different letters are significantly different at the $p<0.05$ level.

The Se concentrations in tissues were significantly higher in rats fed organic form than in these fed inorganic form of Se (Table 1 and 2). In B_6-deficient animals, plasma Se concentrations were elevated, and significant differences were observed in rats fed basal and Se-Met compared with their B_6-adequate counterparts ($p<0.05$). However, erythrocyte Se concentrations were significantly lower ($p<0.05$) in B_6-deficient rats than in B_6-adequate rats.

Since B_6 significantly increased the body weight (Fig. 1), Se concentrations in heart, spleen and liv-

Table 1. Effect of Vitamin B_6 deficiency on Concentrations of Se and Activities of Glutathione Peroxidase in Plasma and Erythrocytes[1-3]

Group	Selenium Concentration		GHS-Px Activity	
	Plasma	Erythrocytes	Plasma	Erythrocytes
	μg/ml		Units[4]	
Basal	0.266±0.019[a]	0.120±0.006[a]	10.8±2.5[a]	90.5± 3.8[a]
Basal+B_6	0.208±0.006[b]	0.135±0.007[a]	15.5±1.6[a]	101.7± 5.8[a]
SeL	0.497±0.017[c]	0.157±0.012[a]	35.1±6.6[b]	97.2± 6.8[a]
SeL+B_6	0.482±0.010[c]	0.363±0.020[b]	30.9±6.0[b]	234.4±10.2[b]
Se-Met	0.594±0.030[d]	0.283±0.017[c]	47.9±1.8[c]	82.2± 7.7[a]
Se-Met+B_6	0.533±0.009[c]	0.429±0.016[d]	32.4±6.2[b]	209.8± 6.4[c]

[1]See figure 1 for experimental details. [2]Results are means±SEM of five rats per group. [3]Means in same column with different letters are significantly different at $p<0.05$ level. [4]Activities of GSH-Px are expressed as nanomole NADPH oxidized/min/mg protein (plasma) or mg Hb (erythrocytes) with H_2O_2 as substrate.

er were significantly lower in B_6-adequate rats than B_6-deficient rats without the addition of Se to the diet. In B_6-deficiency, Se concentrations in muscle, heart, spleen and liver were significantly elevated ($p<0.05$) in rats fed Se-Met compared with their B_6-adequate counterparts, however, there were no significant differences in these tissues (except the heart) of rats fed SeL (Table 2). The heart is only organ in which Se concentration was markedly decreased in B_6-deficient rats fed SeL compared with their B_6-adequate counterparts. Feeding Se-Met generally caused higher amounts of Se to be retained in each of these tissues compared with feeding SeL (Table 2), especially in the muscles, which may be

Table 2. Effect of Vitamin B_6 Deficiency on Concentrations of Se in Muscle, Heart, Spleen, and Liver[1-3]

Group	Muscle	Heart	Spleen	Liver
		(μg/ml)		
Basal	0.08±0.01[a]	0.19±0.01[a]	0.34±0.02[a]	0.36±0.02[a]
Basal+B_6	0.08±0.00[a]	0.16±0.01[b]	0.28±0.02[b]	0.18±0.01[b]
SeL	0.12±0.01[b]	0.30±0.01[c]	0.48±0.02[ce]	1.08±0.02[c]
SeL+B_6	0.14±0.00[b]	0.36±0.01[d]	0.45±0.01[c]	1.12±0.03[c]
Se-Met	0.34±0.02[c]	0.53±0.01[e]	0.67±0.01[d]	1.60±0.03[d]
Se-Met+B_6	0.32±0.01[d]	0.46±0.00[f]	0.52±0.01[e]	1.23±0.03[e]

[1]See figure 1 for experimental details. [2]The results are means±SEM of five rats per group. [3]Means in the same column with different letters are significantly different at the $p<0.05$ level.

related to Se-Met incorporation into general body proteins in place of methionine[6,13].

B_6-deficient rats had higher GSH-Px activity in plasma than their B_6-adequate counterparts, a significant difference ($p<0.05$) in rats fed Se-Met was observed (Table 1). However, B_6-deficient rats had a significant decrease ($p<0.05$) in the activity of GSH-Px in erythrocytes compared with B_6-adequate rats which was in consistent with the changes of Se concentrations in plasma and erythrocytes (Table 1). These results may indicate that Se biopotency for GSH-Px synthesis was reduced dramatically when the diet was deficiency in B_6 regardless of chemical forms of Se. Even though selenite was somewhat less well absorbed than Se-Met[4], this appears not to have a predominant effect on the availability of Se. It seems to be related to the impairment of the active transport of Se across the cell wall or the incorporation of Se into GSH-Px.

Without the addition of Se to the diets, the activities of GSH-Px in muscle and heart had no significant differences between B_6-adequate and B_6-deficient rats (Table 3). A significant increase was found in spleen of B_6-adequate rats, in contrast, a significant decrease was observed in liver of B_6-adequate rats compared with that of B_6-deficient rats. The two Se compounds are equally effective in raising the activity of GSH-Px in B_6-adequate rats but not in B_6-deficient rats. In Se-treated groups, the activities of GSH-Px in muscle, heart and spleen were significantly lower in the rats fed B_6-deficient diet than those fed B_6-adequate diets regardless of the forms of Se, the distinctions were most predominant in the muscle. The hepatic GSH-Px activity was increased by feeding SeL, and the enzyme activity in B_6-deficient rats was similar to that in B_6-adequate rats, whereas in rats fed Se-Met, a significant decrease of the enzyme activity was observed in B_6-deficient rats compared with B_6-adequate rats which results were in consistent with the results obtained by Yasumoto et al[5].

Table 3. Effect of Vitamin B_6 Deficiency on Activities of Glutathione Peroxidase in Heart, Spleen, and Liver[1-4]

Group	Muscle	Heart	Spleen	Liver
Basal	5.9±1.0[a]	83.4± 6.9[a]	173.1±11.4[a]	104.6± 5.4[a]
Basal+B_6	5.6±0.3[a]	117.2± 6.3[a]	222.6± 8.2[be]	39.4± 2.1[b]
SeL	8.6±1.0[a]	213.2±17.9[b]	251.5±18.4[cb]	600.2±30.7[c]
SeL+B_6	39.6±4.8[b]	325.1±34.0[c]	316.7±11.4[d]	590.3±42.5[c]
Se-Met	14.0±2.8[a]	175.8±15.3[b]	194.2±11.2[ae]	506.4±46.3[d]
Se-Met+B_6	32.5±7.2[b]	314.7±31.1[c]	348.0±13.4[d]	624.7±25.9[c]

[1]See figure 1 for experimental details. [2]The results are means±SEM of five rats per group. [3]Means in the same column with different letters are significantly different at the $p<0.05$ level. [4]The activities are expressed as nanomole NADPH oxidized/min/mg protein with H_2O_2 as substrate.

The results of this study indicate that, as measured by GSH-Px activity in liver, Se as Na_2SeLO_3 is more efficient to B_6-deficient rats than Se as Se-Met. However, the effects of Se from Na_2SeLO_3 on the activity of GSH-Px in muscle, heart and spleen have not been observed. Further research is needed to determine whether lowered erythrocyte Se level and GSH-Px activity are related to the impairment of transport process of Se from plasma to erythrocytes observed in B_6-deficient rats fed SeL or Se-Met.

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