Nutrition Research and Practice (2009), 3(3), 208-211
DOI: 10.4162/nrp.2009.3.3.208

Effect of vitamin B_6 deficiency on antioxidative status in rats with exercise-induced oxidative stress*

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Received April 24, 2009; Revised July 15, 2009; Accepted July 20, 2009

Abstract

This study investigated the effect of vitamin B_6 deficiency on antioxidant enzyme activities and lipid profile in rats with exercise-induced oxidative stress. Forty eight rats were fed either a vitamin B_6 deficient diet (B6-) or a control diet (control) for 4 weeks and then subdivided into 3 groups: pre-exercise (PreE); post-exercise (PostE); recess after exercise (recessE). Compared to those of control group, plasma catalase and hepatic cytosol superoxide dismutase (SOD, EC 1.15.1.1) activities of B6- group were lower regardless of exercise. The ratio of reduced glutathione/oxidized glutathione (GSH/GSSG) of B6 - group was lower in PreE and there was no difference between PostE and recessE. The level of malondialdehyde (MDA) of B6- was significantly higher in PreE and PostE. High-density lipoprotein-cholesterol (HDL-C) level of B6- group was lower regardless of exercise. Atherosclerotic index of B6- group was higher in PreE and there was no difference between PostE and recessE. It is suggested that a reduction in antioxidative status caused by vitamin B_6 deficiency may be aggravated under exercise-induced oxidative stress.

Key Words: Vitamin B_6 deficiency, exercise, antioxidative enzymes, oxidative stress

Introduction

Most studies suggested that exercise could be viewed as an effective antioxidant and antiatherogenic therapy. However, evidence is accumulating that strenuous exercise induces an imbalance between free radical production and the body's antioxidant defense systems (Ji, 1999; Lovlin *et al.*, 1991; Maxwell *et al.*, 1993; Sahlin *et al.*, 1991). It has been reported that less the experience one has in training, higher the stress level was gotten (Powers & Hamilton, 1999). The contribution of free radical damage to the development of atherosclerosis is also established (Schwenke, 1998).

Vitamin B_6 seems to be associated in some defense mechanisms especially against lipid peroxidation in tissues, since its deficiency increased this process when animals totally lacked in vitamin B_6 diet (Ravichandran & Selvam, 1990; Ravichandran & Selvam, 1991). Marginal vitamin B_6 contents increased lipid peroxidation and considerably stimulated the activity of glutathione-dependent enzyme (Cabrini *et al.*, 1998). Increased plasma and tissue lipid peroxidation has been reported in rats receiving a vitamin B_6 deficient diet (Benderitter *et al.*, 1996). Pyridoxal 5' phosphate (PLP), the active form of vitamin B_6, is essential as a cofactor for the metabolism of homocysteine to the amino acid, cysteine (Selhub, 1999). Vitamin B_6 deficiency is a risk factor for coronary artery disease by elevated homocysteine levels. In addition, the antioxidative properties of vitamin B_6 have recently been discovered (Jain & Lim, 2001; Matxain, 2006). However, the direct evidence that vitamin B_6 deficiency affects the body antioxidative status with exercise has not been reported. So it is important to study the potential role of vitamin B_6 deficiency on the effects of oxidative stress associated with exercise.

Therefore, the goal of this study was to determine whether vitamin B_6 deficiency has effects on antioxidant enzyme activities and lipid profile under exercise-induced oxidative stress.

Materials and Methods

Experimental diets

Forty eight male weanling Sprague-Dawley rats (Daehanbiolink Co., Korea) were divided into 2 groups: group 1 (control, 24 rats), group 2 (vitamin B_6 deficient, B6-, 24 rats). Rats were received a vitamin-free casein based semi synthetic diet which met AIN-93 recommendation (Reeves, 1997) with the exception of vitamin B_6.

* This study was supported by 2008 Research grant of Institute of Natural Science Research, Duksung Women's University.
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Exercise and sample collection

At the end of week 4, animals in each dietary group were subdivided into 3 exercise groups: pre-exercise (PreE); post-exercise (PostE); recess after exercise (recessE). PreE groups were sacrificed without exercise at the end of week 4. Exercised groups were exercised on a treadmill (10° incline, 0.5-0.8 km/h) with fasting state for 1 hour; animals in the recessE groups were allowed to take a rest for 1 hour after exercise. At the respective time points, animals were sacrificed by decapitation under the light ether anesthesia. Immediately following decapitation, plasma and liver were rapidly removed and stored at -40℃ until analyzed.

Biochemical analysis

The activity of plasma catalase (EC.1.11.1.6) was determined with a commercial kit based on the method of Zamocky (Bioxytech Catalase-520). The activity of superoxide dismutase (SOD, EC 1.15.1.1), the ratio of reduced glutathione and oxidized glutathione, and the level of malondialdehyde were determined in liver cytosol. Liver was homogenized in cold Tris-KCl buffer (0.1 M). The homogenized solution was centrifuged (8,000×g, 4℃, 30 min). The supernatant was then centrifuged (10,000×g, 4℃, 30 min. Again the supernatant was ultra-centrifuged (105,000×g, 4℃, 90 min) and separated the cytosol. SOD activity was determined with a commercial kit based on the method of Nebot (Bioxytech SOD-525). The ratio of reduced glutathione/ oxidized glutathione (GSH/GSSG) was determined with a commercial kit based on the method of Anderson (Bioxytech GSH/GSSG-412). The level of malondialdehyde (MDA) was determined with a commercial kit based on the method of Gerard-Monnier (Bioxytech MDA-586).

Plasma Triglyceride (TG) was analyzed with a commercial kit based on the Trinder method (Youngdong Pharmaceutical Co., Korea). Total cholesterol (TC) was analyzed with a commercial kit based on enzymatic method (Youngdong Pharmaceutical Co., Korea). High-density lipoprotein-cholesterol (HDL-C) was analyzed with a commercial kit based on the same analytical method as total cholesterol after the precipitation of very low-density lipoprotein-cholesterol (VLDL-C), low-density lipoprotein-cholesterol (LDL-C) and chylomicron with polyethyleneglycol (International Reagent Co., Japan). Atherosclerotic index was calculated as (TC-HDL-C)/HDL-C.

Pyridoxal 5'-phosphate (PLP) was measured by HPLC method (Kimura *et al.*, 1996) which was modified as follows: The mobile phase (0.1 M potassium dihydrogen phosphate containing 0.1 M sodium perchlorate, 0.5 g/l sodium bisulfite, pH 3) was pumped at a flow rate of 1.0 ml/min into the column (μBondpack ODS column, 3.9×300 mm, 10 μm porous packing, C_{18}, Waters). Tissue samples were homogenized in cold sodium phosphate buffer (80 mM, pH 7.4). Aliquots of the tissue homogenates and plasma were added to perchrolic acid (1 M) and allowed to sit for one hour to release PLP from protein. This mixture was then centrifuged (18000×g, 4℃, 15 min) and the supernatants were removed. Fifty μl aliquot of supernatant was loaded in the sample loop and then injected onto the column. Samples for vitamin B_6 analysis were prepared under yellow fluorescent lighting to prevent photodegradation of the vitamers.

Statistical analysis

All data were subjected to the analysis of variance and tested for significant differences by Duncan's multiple range tests (SAS Institute, Cary, NC). A p value < 0.05 was considered to be significant. The significance of difference between control group and B_6- group was tested using independent two-sample t-test at $P < 0.05$.

Results

Table 1 demonstrates that the feed efficiency ratio (FER) and the final body weight of B6- were significantly lower than those of the control group. Plasma PLP concentration of B6- group was also significantly lower than those of the control group. Thus, it was considered that rats fed B6- diets became deficient in vitamin B_6 by the 4th week.

Table 2 demonstrates the effect of vitamin B_6 deficiency on catalase activities. Compared to those of control group, the catalase activity of B6- group was significantly lower regardless of exercise. SOD activity of B6- group was also lower regardless of exercise. SOD activity of B6- group was decreased with exercise and was significantly lower than that of control groups in post-exercise and recess after exercise. Compared to those of control group, GSH/GSSG ratio was significantly lower in vitamin B_6 deficient rats with pre-exercise. However, there was no significant difference between control and B6- groups in post-exercise and recess after exercise because GSH/GSSG ratio of control group was decreased with exercise but those of B6- groups was not significantly changed in post-exercise and recess after exercise. Table 3 demonstrates the effect of vitamin B_6 deficiency on MDA levels. Compared to those of control group, MDA levels were significantly higher in vitamin B_6 deficient rats in pre- and post-exercise and there was no difference between control and B6- groups in recess after exercise. Table 4

Table 1. The final body weight, feed efficiency ratio and the concentration of plasma pyridoxal-5-phosphate

	Control	B6-	*t*-test
Initial BW (g)	70.2 ± 2.7	70.2 ± 2.7	NS
Final BW (g)	299 ± 43	189 ± 12	*
FER	0.35 ± 0.10	0.27 ± 0.03	*
PLP (pmol/ml)	340 ± 157	167 ± 50	*

Control: control diet, B6-: vitamin B_6 deficient diet
BW: body weight, FER: food efficiency ratio =total weight gain/total food intake, PLP: pyridoxal-5-phosphate
* Significant difference between control group and B6-group, $P < 0.05$ (*t*-test), NS: no significant difference between control group and B6-group

Table 2. The effect of vitamin B_6 deficiency on the activity of plasma catalase and liver superoxide dismutase and the ratio of reduced glutathione and oxidized glutathione

		PreE	PostE	recessE
Catalase (U/mg protein)	Control	22.14 ± 0.91[a]	10.44 ± 1.21[b]	9.68 ± 0.79[b]
	B6-	8.31 ± 1.70	8.16 ± 0.78	8.84 ± 1.26
	t-test	*	*	*
SOD (U/mg protein)	Control	508.00 ± 33.50	479.63 ± 52.85	504.62 ± 21.74
	B6-	437.64 ± 66.32[a]	307.09 ± 30.97[b]	355.26 ± 28.84[b]
	t-test	*	*	*
GSH/GSSG	Control	20.66 ± 4.60[a]	7.76 ± 2.89[b]	12.06 ± 4.59[b]
	B6-	10.59 ± 2.58	7.82 ± 0.97	8.26 ± 1.46
	t-test	*	NS	NS

Control: control diet, B6-: vitamin B_6 deficient diet
PreE: pre-exercise, PostE: post-exercise, recessE: recess after exercise
SOD: superoxide dismutase in liver cytosol, GSH/GSSG: the ratio of reduced glutathione and oxidized glutathione in liver cytosol
Values in the same row with different superscript symbols (a, b) is significantly different, $P<0.05$, NS: no significant difference among exercised groups
* Significant difference between control group and B6-group, $P<0.05$ (*t*-test), NS: no significant difference between control group and B6-group

Table 3. The effect of vitamin B_6 deficiency on MDA level in liver

		PreE	PostE	recessE
MDA (nmol/mg protein)	Control	17.95 ± 7.83[a]	22.44 ± 5.11[b]	20.98 ± 4.89[b]
	B6-	23.98 ± 1.91[a]	28.23 ± 7.90[b]	23.34 ± 5.67[a]
	t-test	*	*	NS

Control: control diet, B6-: vitamin B_6 deficient diet
PreE: pre-exercise, PostE: post-exercise, recessE: recess after exercise
MDA: malondialdehyde in liver cytosol
Values in the same row with different superscript symbols (a, b) are significantly different, $P<0.05$.
* Significant difference between control group and B6-group, $P<0.05$ (*t*-test), NS: no significant difference between control group and B6-group

Table 4. The effect of vitamin B_6 deficiency on plasma lipid profile

		PreE	PostE	recessE
TG (mg/dl)	Control	195.75 ± 55.73[a]	92.62 ± 38.04[b]	98.75 ± 28.45[b]
	B6-	91.30 ± 29.30[a]	63.25 ± 34.44[b]	42.25 ± 18.63[c]
	t-test	*	*	*
TC (mg/dl)	Control	83.12 ± 12.5[a]	115.67 ± 24.78[b]	129 ± 28.07[b]
	B6-	78.20 ± 8.71	77.13 ± 7.53	75.13 ± 13.73
	t-test	NS	*	*
HDL-C (mg/dl)	Control	33.96 ± 15.86	29.84 ± 5.28	28.81 ± 10.48
	B6-	15.63 ± 5.55	19.98 ± 9.04	18.357 ± 9.08
	t-test	*	*	*
Atherosclerotic index	Control	2.14 ± 1.78[a]	3.34 ± 0.31[b]	3.59 ± 2.00[b]
	B6-	4.68 ± 2.12	3.65 ± 2.20	3.75 ± 1.78
	t-test	*	NS	NS

Control: control diet, B6-: vitamin B_6 deficient diet
PreE: pre-exercise, PostE: post-exercise, recessE: recess after exercise
TG: Triglyceride, TC: total cholesterol, HDL-C: high density lipoprotein cholesterol, Atherosclerotic index = (TC-HDL-C)/HDL-C
Values in the same row with different superscript symbols (a, b) is significantly different, $P<0.05$, NS: no significant difference among exercised groups
* Significant difference between control group and B6-group, $P<0.05$ (*t*-test), NS: no significant difference between control group and B6-group

demonstrates the effect of vitamin B_6 deficiency on plasma lipid profile. Compared to those of control group, the triglyceride level was significantly low in vitamin B_6 deficient rats regardless of exercise and the tendency of decrease in post-exercise and recess after exercise was similar in both control and B6- groups. HDL-C level was significantly low in vitamin B_6 deficient rats regardless of exercise and the tendency of no change in post-exercise and recess after exercise was similar in both control and B6- groups. Compared to those of control group, atherosclerotic index was significantly higher in vitamin B_6 deficient rats in pre-exercise. However, there was no significant difference between control and B6- groups in post-exercise and recess after exercise.

Discussion

This study demonstrated that a reduction in antioxidative status caused by vitamin B_6 deficiency may be aggravated under exercise-induced oxidative stress. At various points during the study, the antioxidative status in rats was evaluated using the ratios of GSH/GSSG and the activities of catalase and SOD as a direct measure and the level of MDA and lipid profile as an indirect, long-term measure. The vitamin B_6 deficiency in rats was verified by the lowered plasma PLP levels as a direct measure and lowered body weight and FER as an indirect, long-term measure.

The hypothesis that vitamin B_6 deficiency cannot react effectively to stress and decreases the antioxidative status was verified by results from two different measurements. First, GSH/GSSG ratio was significantly lower in vitamin B_6 deficient rats in pre-exercise. This decreased activity of antioxidant enzymes has been also reported in vitamin B_6 deficiency (Bordoni *et al.*, 2006; Selvam & Ravichandran, 1993). Decreased GSH/GSSG ratio suggests a degraded antioxidant protection, which may have contributed to the higher exercise-induced ROS following vitamin B_6 deficient diet. There was no significant differences between control group and vitamin B_6 deficient group in post-exercise and recess after exercise because GSH/GSSG ratio of control group was decreased with exercise but those of vitamin B_6 deficient groups was not significantly changed in post-exercise and recess after exercise. Therefore it is assumed that vitamin B_6 deficiency induced an increase of plasma glutathione peroxidase activity and a decrease of plasma total antioxidant status under pre-exercise conditions and was accompanied by a decreased ratio of reduced glutathione and oxidized glutathione. It is generally reported that glutathione peroxidase activity after regular exercise training is increased in rats (Powers *et al.*, 1999) and resting GSH/GSSG levels is increased 61% following isometric exercise training in humans (Peters *et al.*, 2006). It is also reported that antioxidant nutrient status and exercise training have an interactive effect on oxidative stress and antioxidant enzyme activities (Benderitter *et al.*, 1996; Chang *et al.*, 2007). Although, compare to control group, the catalase activity of vitamin B_6 deficient group was significantly lower regardless of exercise, this difference was decreased in post-exercise and recess after exercise because exercise-induced

oxidative stress induced the decrease of plasma catalase activity in control group but exercise-induced oxidative stress did not affect the catalase activity in vitamin B_6 deficient group. Exercise increases oxygen consumption and generation of reactive oxygen species such as superoxide and hydrogen peroxide. The SOD activity of control group remained stable with exercise-induced oxidative stress but the SOD activity of vitamin B_6 deficient groups was decreased with exercise and was significantly lower in post-exercise and recess after exercise. Thus, it is suggested that vitamin B_6 deficient rats did not react effectively to stress and decreased antioxidant enzymes activity in this study.

Second, an increased susceptibility to lipid peroxidation is facilitated by the decreased activities of antioxidant enzymes. Previous studies reported that the concentration of thiobarbituric acid reactive substances (TBARS) in liver was high in vitamin B_6 deficient rats (Benderitter *et al.*, 1996) and the liver glutathione concentration was increased in rats fed excess vitamin B_6 (Mahfouz & Kummerow, 2004). Increased susceptibility to lipid peroxidation in rat liver and heart (Cabrini *et al.,* 1998), rat plasma (Ravichandran & Selvam, 1991) and cells of Fusarium species (Kayali & Tarhan, 2006) was also reported. Because the MDA level in this study was significantly higher in vitamin B_6 deficient rats in pre- and post-exercise and tended to be higher in recess after exercise although the difference was not significant, it is assumed that vitamin B_6 deficiency leads to an increase of lipid peroxidation in rats.

Compared to that of control group, atherosclerotic index was significantly higher in vitamin B_6 deficient rats in pre-exercise. However, there was no significant difference between control group and vitamin B_6 deficient group in post-exercise and recess after exercise because atherosclerotic index of control group was increased in post-exercise and recess after exercise but that of vitamin B_6 deficient group was not significantly changed with exercise. Also, HDL-C level was significantly low in vitamin B_6 deficient rats regardless of exercise. Thus, it is suggested that vitamin B_6 deficiency has a negative effect on atherosclerotic index but does not aggravate it further under exercised induced oxidative stress.

Therefore, despite the many uncertainties regarding the mode of action, these results suggest that vitamin B_6 deficient animal do not react effectively to oxidative stress and a reduction in antioxidative status caused by vitamin B_6 deficiency may be aggravated under exercise-induced oxidative stress.

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