Effect of vitamin B₆ deficiency on antioxidative status in rats with exercise-induced oxidative stress

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Abstract

This study investigated the effect of vitamin B₆ deficiency on antioxidant enzyme activities and lipid profile in rats with exercise-induced oxidative stress. Forty eight rats were fed either a vitamin B₆ 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 B₆ deficiency may be aggravated under exercise-induced oxidative stress.

Key Words: Vitamin B₆ 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 B₆ 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 B₆ diet (Ravichandran & Selvam, 1990; Ravichandran & Selvam, 1991). Marginal vitamin B₆ 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 B₆ deficient diet (Benderitter et al., 1996). Pyridoxal 5’ phosphate (PLP), the active form of vitamin B₆, is essential as a cofactor for the metabolism of homocysteine to the amino acid, cysteine (Sellub, 1999). Vitamin B₆ deficiency is a risk factor for coronary artery disease by elevated homocysteine levels. In addition, the antioxidative properties of vitamin B₆ have recently been discovered (Jain & Lim, 2001; Matxain, 2006). However, the direct evidence that vitamin B₆ deficiency affects the body antioxidative status with exercise has not been reported. So it is important to study the potential role of vitamin B₆ deficiency on the effects of oxidative stress associated with exercise.

Therefore, the goal of this study was to determine whether vitamin B₆ 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 B₆ 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 B₆.
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 °C 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°C, 30 min). The supernatant was then centrifuged (10,000×g, 4°C, 30 min). Again the supernatant was ultra-centrifuged (105,000×g, 4°C, 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.

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 B6 deficient diet

Table 2 demonstrates the effect of vitamin B6 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 B6 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 B6 deficiency on MDA levels. Compared to those of control group, MDA levels were significantly higher in vitamin B6 deficient rats in pre- and post-exercise and there was no difference between control and B6- groups in recess after exercise. Table 4

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 B6 by the 4th week.

Table 2 demonstrates the effect of vitamin B6 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 B6 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 B6 deficiency on MDA levels. Compared to those of control group, MDA levels were significantly higher in vitamin B6 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](image-url)
was significantly low in vitamin B6 deficient rats regardless of trough incidence. Compared to those of control group, atherosclerotic index was significantly higher in vitamin B6 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 B6 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 B6 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 B6 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 B6 deficient rats in pre-exercise. This decreased activity of antioxidant enzymes has been also reported in vitamin B6 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 B6 deficient diet. There was no significant differences between control group and vitamin B6 deficient group in post-exercise and recess after exercise because GSH/GSSG ratio of control group was decreased with exercise but those of vitamin B6 deficient groups was not significantly changed in post-exercise and recess after exercise. Therefore it is assumed that vitamin B6 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 B6 deficient group was significantly lower regardless of exercise, this difference was decreased in post-exercise and recess after exercise because exercise-induced

demonstrates the effect of vitamin B6 deficiency on plasma lipid profile. Compared to those of control group, the triglyceride level was significantly low in vitamin B6 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 B6 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 B6 deficient rats in pre-exercise. However, there was no significant difference between control and B6- groups in post-exercise and recess after exercise.

Table 2. The effect of vitamin B6 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 * | 10.44 ± 2.13 * | 9.68 ± 0.79 * |
| 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 | 20.60 ± 4.60 * | 7.76 ± 2.89 * | 12.06 ± 4.59 * |
| B6-     | 10.59 ± 2.58  | 7.82 ± 0.97  | 8.26 ± 1.46  |
| t-test  | *           | NS          | NS           |

Control: control diet, B6-: vitamin B6 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 B6 deficiency on MDA level in liver

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

Control: control diet, B6-: vitamin B6 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 B6 deficiency on plasma lipid profile

|         | PreE       | PostE     | recessE      |
|---------|------------|-----------|--------------|
| TG (mg/dl) |
| Control | 195.75 ± 55.73  | 92.62 ± 38.04  | 98.75 ± 28.45 |
| B6-     | 91.30 ± 29.30 a | 63.25 ± 34.44 b | 42.25 ± 18.63 b |
| t-test  | *           | *           | *            |
| TC (mg/dl) |
| Control | 83.12 ± 12.5  | 115.67 ± 24.76 | 129 ± 28.07 |
| B6-     | 78.20 ± 6.71  | 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  | *            | NS          | NS           |

Control: control diet, B6-: vitamin B6 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
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 B₆ 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 B₆ deficient groups was decreased with exercise and was significantly lower in post-exercise and recess after exercise. Thus, it is suggested that vitamin B₆ 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 B₆ deficient rats (Benderitter et al., 1996) and the liver glutathione concentration was increased in rats fed excess vitamin B₆ (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 B₆ 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 B₆ deficiency leads to an increase of lipid peroxidation in rats.

Compared to that of control group, atherosclerotic index was significantly higher in vitamin B₆ deficient rats in pre-exercise. However, there was no significant difference between control group and vitamin B₆ 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 B₆ deficient group was not significantly changed with exercise. Also, HDL-C level was significantly low in vitamin B₆ deficient rats regardless of exercise. Thus, it is suggested that vitamin B₆ 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 B₆ deficient animal do not react effectively to oxidative stress and a reduction in antioxidative status caused by vitamin B₆ deficiency may be aggravated under exercise-induced oxidative stress.

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