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High Intensity Exercise Induced a Redistribution of Pyridoxal 5'-Phosphate Levels with Different Vitamin B_6 Status in Rats*

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ABSTRACT

The purpose of this study was to compare the changes in PLP concentrations induced by regular, moderate, and abrupt, high-intensity exercise in the plasma and tissues of vitamin B_6 deficient and normal rats. Forty-eight rats were fed either a vitamin B6 deficient (−B6) diet or a normal (+B6) diet for 5 weeks and were subdivided into 4 groups:non-exercise (NE) group; regular, moderate-intensity exercise (RME) group; abrupt, high-intensity exercise (AIE) group; abrupt, high-intensity exercise and recuperation (IER) group. The RME group was exercised on treadmill (10°, 0.5 − 0.8 km/h) for 20 minutes every day throughout the experimental period. The AIE group was exercised on a treadmill (10°, 0.5 − 0.8 km/h) for 2 hours just before sacrifice at the end of 5th week on the diet and the IER group was recuperated for three days on the diet after being exercised like the AIE group. Pyridoxal 5'-phosphate (PLP) levels were compared in the plasma, liver and skeletal muscle of the rats. Plasma PLP concentration tended to decrease in −B6 rats and tended to increase in +B6 rats with AIE. Plasma PLP concentrations in both +B6 and −B6 rats did not change with RME. Liver PLP concentration significantly increased in −B6 rats, showed no change in +B6 rats with AIE and no change in both −B6 and +B6 rats with RME. Muscle PLP concentration decreased in +B6 rats and showed no change in −B6 rats with AIE. Muscle PLP concentrations in both +B6 and −B6 rats did not change with RME. Plasma PLP, liver PLP and muscle PLP concentration of IER returned to those of NE in both +B6 and −B6 rats. These results suggest that changes in PLP concentration in plasma, liver and muscle occur with exercise and are affected by exercise intensity and vitamin B_6 status. These changes may be due to interorgan redistribution of PLP.

KEY WORDS: vitamin B6 status, exercise, recuperation, pyridoxal 5'-phosphate (PLP).

INTRODUCTION

Physical activity is associated with an increased energy requirement and the energy used for exercise in animals is derived predominantly from carbohydrates and fat, including coenzyme reactions, which frequently need vitamins as essential components. It has been reported indirectly that vitamin B_6 may be involved in this energy supply. Pyridoxal 5'-phosphate (PLP), the active form of vitamin B_6, acts as an integral part of glycogen phosphorylase (EC 2.4.1.1.), which catalyzes the breakdown of glycogen.[1] PLP is a cofactor for aminotransferase, which catalyzes the conversion of certain amino acids to glucose.[2] It can also be expected that regular exercise requires more vitamin B_6 due to increased anabolic activity. PLP is also required in biosynthesis of carnitine, which acts as a carrier of fattyacyl group across the mitochondrial membrane.[3]

It has been reported that plasma PLP concentration and the erythrocyte aspartateaminotransferase activation coefficient are increased in exercising subjects under normal vitamin B_6 status.[4-7] Two different hypotheses have been presented. One suggests that that inter-organ transfer of vitamins for coenzymatic use occurs.[4,8,9] Under this hypothesis, the coenzyme would be needed either in the liver for aminotransferase reactions, providing amino acid skeletons for gluconeogenesis or in the muscle to facilitate an accelerated rate of aminotransferase. An alternative hypothesis[10] suggests that the rises in plasma PLP during exercise are more likely a concomitant event accompanying temporary protein shifts into the blood than an adaptive event that facilitates fuel provision during endurance exercise. It is also known that energy source for exercise is different with duration and intensity of exercise and PLP may be involved in this energy supply. However, there have been little studies of changes in PLP levels with different intensity of exercise. Also, the scheme of the change in PLP levels may be different in vitamin B_6 deficient state because the reservoir[11] of vitamin B_6 is not sufficient and less PLP may be mobilized compared to the normal state.

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Thus, the aim of this study was to compare the changes of PLP concentrations induced by regular, moderate- and abrupt, high-intensity exercise in plasma and tissues of vitamin B_6-deficient and normal rats.

MATERIALS AND METHODS

1. Experimental animals and diets.

Forty eight weanling male Sprague-Dawley rats of 40–60 g were fed either a vitamin B_6-deficient diet or normal diet. Normal diet was the vitamin-free casein-based semisynthetic diet which met AIN-76 recommendations.[12][13] The composition of vitamin B_6 deficient diet was the same as that of normal diet except that vitamin B_6 was omitted. The rats were subdivided into 4 groups: non-exercise (NE) group; regular, moderate-intensity exercise (RME) group; abrupt, high-intensity exercise (AIE) group; abrupt, high-intensity exercise and recuperation (IER) group. The normal rats were pair-fed against the intake of the vitamin B_6 deficient rats to minimize variations due to differences in the amount of diet consumption. Each group was fed their respective diet for 5 weeks.

2. Exercise and sample collection

The RME group was exercised on a treadmill (JKEXER, 618A, KOREA; 10°, 0.5–0.8 km/h) for 20 minutes every day throughout the experiment period (5 weeks). The AIE group was exercised on a treadmill (10°, 0.5–0.8 km/h) for 2 hours just before sacrifice at the end of 5th week on the diet and IER group was recuperated three days on the diet after being exercised like the AIE group. The animals were sacrificed by decapitation under light ether anesthesia after 16 hours of fasting. Immediately following decapitation, blood was collected in heparinized tubes and centrifuged to separate the plasma. Liver and skeletal muscle was rapidly removed. The plasma and tissues were stored at −40℃ until analyzed.

3. Biochemical analysis

Pyridoxal 5'-phosphate (PLP) was measured by the HPLC method,[14] which was modified as follows: Mobil 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 porus packing, C18, 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 supernatent 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.[15]

3. Statistical analysis

All data were subjected to an analysis of variance and tested for significant differences by Duncan's multiple range test (SAS Institute, Cary, NC). A p value < 0.05 was considered to be significant.

RESULTS

The vitamin B_6 status of the rats was evaluated using body weight as an indirect, long-term measure and plasma and tissue PLP concentration as a direct measure. The effect of various exercise intensities and vitamin B_6 deficiencies on body weight and food efficiency ratio is shown in Table 1. The mean body weight and food efficiency ratio of the vitamin B_6 deficient groups (non-exercise; regular, moderate-intensity exercise; abrupt, high-intensity exercise; abrupt, high-intensity exercise and recuperation) at week 5 were significantly lower than those of the normal groups, although they were pair-fed. The concentrations of PLP in the plasma, liver and muscle of vitamin B_6 deficient rats were significantly lower than those of normal rats (Table 2). Thus, vitamin B_6 deficient rats were severely vitamin B_6 deficient by the 5th week of the study.

Fig. 1 shows the effect of vitamin B_6 deficiency and ex-

Table 1. The effects of vitamin B_6 deficiency and exercise intensity on body weights and food efficiency ratio[1][2]

	+B6[3]				−B6			
	NE	RME	AIE	IER	NE	RME	AIE	IER
Body weight (g)	164 ± 5[a]	162 ± 2[a]	160 ± 5[a]	167 ± 6[a]	140 ± 17[b]	140 ± 13[b]	140 ± 16[b]	140 ± 20[b]
Food efficiency ratio	0.29 ± 0.02[a]	0.30 ± 0.01[a]	0.29 ± 0.04[a]	0.30 ± 0.06[a]	0.25 ± 0.04[b]	0.26 ± 0.04[b]	0.25 ± 0.04[b]	0.24 ± 0.04[b]

1) Vaues are mean ± SEM, n = 6

2) Within a given row, those values with different superscripts are significantly different ($p < 0.05$)

3) +B6 = normal diet; −B6 = vitamin B_6 deficient diet; NE = non-exercise group, RME = regular, moderate-intensity exercise group, AIE = abrupt, high-intensity exercise group; IER = abrupt, high-intensity exercise and recuperation group

ercise intensity on the mean PLP concentrations in plasma. In the vitamin B_6 deficient rats, the mean plasma PLP concentration did not change with regular, moderate-intensity exercise and tended to decrease with abrupt, high-intensity exercise while in the normal rats, the mean plasma PLP concentration did not change with regular, moderate-intensity exercise and tended to be increase with abrupt, high-intensity exercise. After the abrupt, high-intensity exercised animals were recuperated for three days, the mean plasma PLP concentration returned to that of non-exercised animals in vitamin B_6 deficient rats and in normal rats.

Fig. 2 shows the effect of vitamin B_6 deficiency and exercise intensity on the mean PLP concentrations in the liver. With abrupt, high-intensity exercise, the mean liver PLP concentration significantly increased in the vitamin B_6 deficient rats but the concentration in normal rats did not change. The mean liver PLP concentration did not change in both the vitamin B_6 deficient rats and normal rats with regular exercise. After recuperating for three days, PLP concentration in both the vitamin B_6 deficient rats and normal rats returned to that of non-exercise animals.

The effect of exercise intensity on the mean muscle PLP concentrations with and without vitamin B_6 deficiency is shown in Fig. 3. With regular exercise, the mean muscle PLP concentration of vitamin B_6 deficient rats and normal rats did not change. With abrupt, high-intensity exercise, the mean muscle PLP concentrations in the vitamin B_6 deficient rats did not change. In normal rats, the mean muscle PLP concentration decreased significantly with abrupt, high-intensity exercise. After recuperating for three days, PLP levels of both groups returned to levels comparable to those of the non-exercise animals.

Table 2. The effect of vitamin B_6 deficiency on the concentration of pyridoxal 5'-phosphate in plasma, liver and muscle[1)2)]

	+B6[3)]	−B6
Blood (nmol/L)	527.8 ± 133.2[a]	254.0 ± 55.9[b]
Liver (nmol/g)	24.6 ± 0.93[a]	9.59 ± 3.11[b]
Muscle (nmol/g)	19.88 ± 2.87[a]	7.48 ± 0.33[b]

1) Vaues are mean ± SEM, n = 24
2) Within a given row, those values with different superscripts are significantly different ($p < 0.05$)
3) +B6 = normal diet; −B6 = vitamin B_6 deficient diet

DISCUSSION

This study demonstrated that exercise resulted in redistribution of PLP and is most evident in normal rats. The evidence of PLP redistribution is based on the changes of PLP concentration in plasma and tissues with abrupt, high-intensity exercise. The results of this study are consistent with changes in plasma PLP concentration observed following exercise in humans.[4)5)]

In the normal rats, abrupt, high-intensity exercise tended to increase the PLP concentration in plasma and significantly decrease it in muscle. It is reported that skeletal

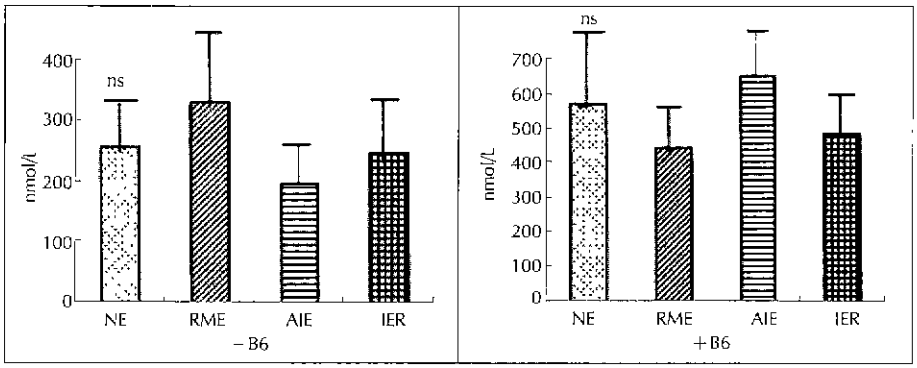

Fig. 1. The effect of vitamin B_6 deficiency and exercise intensity on the pyridoxal 5'-phosphate concentrations in plasma (Ns is not significantly different ($p < 0.05$). +B6 normal diet, −B6 vitamin B_6 deficient diet, NE non-exercise group, RME regular, moderate-intensity exercise group, AIE abrupt, high-intensity exercise group IER abrupt, high-intensity exercise and recuperation group).

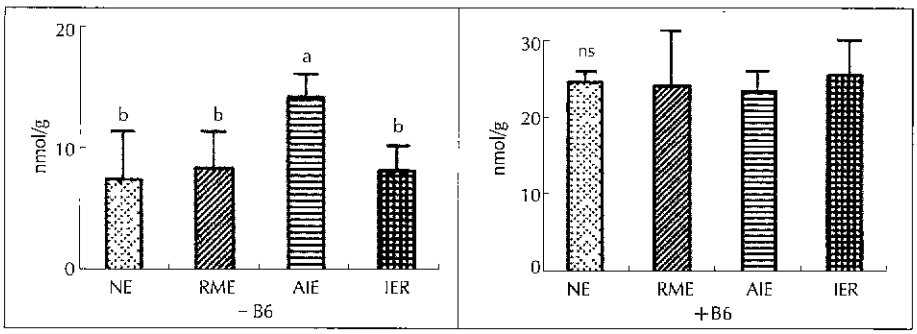

Fig. 2. The effect of vitamin B_6 deficiency and exercise intensity on the pyridoxal 5'-phosphate concentrations in liver (Ns is not significantly different ($p < 0.05$) +B6: normal diet; −B6: vitamin B_6 deficient diet; NE: non-exercise group, RME: regular, moderate-intensity exercise group, AIE: abrupt, high-intensity exercise group, IER: abrupt, high-intensity exercise and recuperation group).

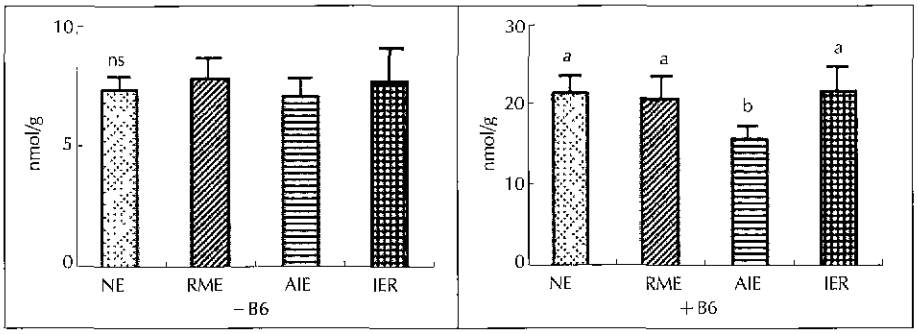

Fig. 3. The effect of vitamin B_6 deficiency and exercise intensity on the pyridoxal 5'-phosphate concentrations in muscle (Each bar with different letters is significantly different ($p < 0.05$). Ns is not significantly different ($p < 0.05$). +B6: normal diet −B6: vitamin B_6 deficient diet NE: non-exercise group, RME: regular, moderate-intensity exercise group, AIE abrupt, high-intensity exercise group, IER: abrupt, high-intensity exercise and recuperation group).

muscle glycogen phosphorylase serves as a storage depot for vitamin B_6 and that the total amount of glycogen phosphorylase in muscle decreased when caloric deficit was introduced[11] and fasting increased the PLP concentration in plasma in vivo.[16,17] Leklem[6] extended this concept to exercise, positing that exercise induces a transient starvation-like metabolic state followed by PLP efflux out of muscle and it is reported that the substrate utilization during exercise is like a fasting state in vivo.[18,19] Thus, the source of increased PLP in the plasma of the normal rats with abrupt, high-intensity exercise may be muscle glycogen phosphorylase.

In vitamin B_6 deficient rats, as a result of the vitamin B_6 deprivation, less PLP would be synthesized in the liver and stored in muscle. This deprivation effect might be greater than any PLP increase due to high-intensity exercise. It even increased in the livers of vitamin B_6 deficient rats in this study. Thus, the increased hepatic need for PLP in vitamin B_6 deficient rats would result in a decrease in the concentration of PLP in the plasma of vitamin B_6 deficient rats with high intensity exercise. These results are consistent with the report that the vitamin B_6 status and vitamin B_6 intake of high performance athletes was lower than that of untrained individuals.[20] If the exercise induced PLP rise is related to increased reaction rates in either the liver or muscle, then it is possible that the response of PLP concentration to exercise may change at different exercise intensities. The present study showed that the PLP concentration of plasma, liver and muscle did not change with regular, moderate intensity exercise in normal rats and vitamin B_6 deficient rats while PLP concentration tended to increase in plasma and significantly decrease in muscle with high intensity exercise in normal rats. This is in contrast to the report that exercise intensity had no effect on the magnitude or rate of increase for PLP.[10] With regular, moderate intensity exercise, caloric deficit was not introduced in the animals, and may have been no increase in PLP efflux from muscle. Following recuperation after abrupt, high intensity exercise, PLP concentration of plasma, liver, and muscle of both normal rats and vitamin B_6 deficient rats returned to that of non-exercise levels. These points support the PLP redistribution hypothesis with abrupt, high-intensity exercise.

In summary, this study shows that the changes of PLP concentrations in plasma, liver and muscle occur with exercise and are affected by exercise intensity and vitamin B_6 status. In the normal rats, abrupt, high-intensity exercise tended to increase the PLP concentration in plasma and significantly decrease in muscle. The source of increased PLP in the plasma may be muscle glycogen phosphorylase. In vitamin B_6 deficient rats, as a result of the vitamin B_6 deprivation, less PLP would be synthesized in the liver and stored in muscle, and this would result in a decrease in the concentration of PLP in the plasma of vitamin B_6 deficient rats. These changes may be due to a redistribution of PLP between plasma and tissues for coenzymatic use rather than a concomitant event accompanying temporary protein shifts into blood to facilitate fuel provision during endurance exercise.

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