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The Effect of *Allium* Vegetable Intake on the Redistribution of Pyridoxal 5'-phosphate Levels in Exercising Rats*

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This study investigated the effect of allium vegetable intake on the storage and utilization of energy substrates before, during, and after exercise in tissues of rats. Ninety rats were fed either a control diet or a diet with added *allium sativum* (AS), *allium cepa* (AC), *allium fistulosum* (AF), or *allium tuberosum* (AT) for 4 weeks and then subdivided into 3 groups: before-exercise (BE) during-exercise (DE) after-exercise (AE). The DE group exercised on treadmill for 1 hour just before being sacrificed at the end of the 4th week of the dietary treatment. Rats in the AE group were allowed to recuperate for 2 hours after being exercised like the DE group. Pyridoxal 5'-phosphate (PLP) levels were compared in plasma, liver and skeletal muscle of rats. There was no difference between AS animals and control animals in plasma PLP levels regardless of exercise. The plasma PLP levels of AC animals were higher than those of control animals before exercise but this PLP was decreased with exercise and lower than that of control animals after exercise. The plasma PLP levels of AF animals were higher than thoseof control animals during exercise but there was no difference before and after exercise. The plasma PLP levels of AT animals were higher than those of control animals regardless of exercise. Compared to those of control rats, the PLP levels of liver and muscle were significantly lower in AS, AC, AF and AT rats before exercise. The levels of liver PLP were significantly decreased in control rats while not changed in AS, AC, AF and AT rats during exercise. The levels of liver PLP tended to decrease in AS, AC and AF rats after exercise. The levels of muscle PLP were significantly decreased in control rats, while not changed in AS, AC and AF rats during exercise. The levels of muscle PLP were decreased in control rats but not changed in AS, AC and AF rats after exercise. Thus, it is suggested that the changes of PLP concentrations in plasma and tissues induced by exercise are affected by *allium* vegetable diet and demonstrated that *allium* vegetable intake induced an alteration in the redistribution of PLP among tissues.

Key word: *Allium* sativum, *Allium cepa*, *Allium fistulosum*, *Allium tuberosum*, Exercise, PLP

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INTRODUCTION

Physical activity is associated with an increased energy requirement and the energy used for exercise in animals is derived predominantly from carbohydrate and fat, including the coenzyme reactions, which frequently need vitamins as essential components. It has been reported indirectly that vitamin B6 may be involved in this energy supply. Pyridoxal 5'-phosphate (PLP), active form of vitamin B6, 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 needs more vitamin B6 due to increased anabolic processes. PLP is also required in the biosynthesis of carnitine which acts as a carrier of fatty acyl group across the mitochondrial membrane.[3]

It has been also reported indirectly that *allium* vegetables may be involved in this fuel metabolism. *Allium* vegetables have been consumed for their putative nutritional and health benefits for centuries. Recent studies have shown that *allium* vegetables contain active compounds regulating many metabolic processes. Organosulfur compounds in *allium* vegetables have cholesterol and lipid lowering effect.[4-8] Also, flavonoids found in *allium* vegetables have been reported to play a role in preventing oxidative damages induced by active oxygen radicals in living systems and non-enzymatic lipid oxidation.[9-15] Accumulating evidences have shown

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that untrained and strenuous exercise induces an imbalance between free radical production and the body's antioxidant defense systems. Given that high intensity exercise can increase free radical production, antioxidant supplements may offer benefits during prolonged aerobic activity and reduce fatigue.[16-20] However, the direct evidence which *allium* vegetables, free radical scavengers affect PLP levels during exercise and affect the endurance capacity of muscle power has not been reported. Therefore, the aim of this study was to investigate the effect of *allium* vegetable diet on the redistribution of PLP levels during exercise *in vivo*.

MATERIALS AND METHODS

1. Experimental Animals and Diets

Ninety male Sprague-Dawley rats (Daehanbiolink Co., Korea) weighing 95-105 g were fed either the control diet or one of the *allium* supplemented diets with either *allium* sativum (AS, garlic), *allium cepa* (AC, onion), *allium fistulosum* (AF, spring onion), or *allium tuberosum* (AT, Chinese chives). The control diet was vitamin-free casein based semisynthetic diet which met AIN-93 recommendation.[21] The composition of *allium* vegetable diet was the same as that of control diet except for the amount of cellulose. The diets were adjusted to be isocaloric by adding 10% cellulose to the control diet and 10% *allium* vegetable powder to the *allium* vegetable diets (Table 1). Thus, all experimental diets contained 14% protein, 4% fat, 67% carbohydrates, 10% fiber and 3600 Kcal/kg by weight. *Allium* vegetable powders were purchased at a local market (Bumi Food Co., Korea). Animals received 10% *allium* vegetable diets or control diet for 4 weeks.

Table 1. Composition of diet[1)]

Ingredient (g/kg)	CONT[2)]	AS	AC	AF	AT
Casein	140	140	140	140	140
Sucrose	100	100	100	100	100
Soybean Oil	40	40	40	40	40
tert-butylhydroquinone	0.008	0.008	0.008	0.008	0.008
Cornstarch	465.7	465.7	465.7	465.7	465.7
Dyetrose	155	155	155	155	155
Cellulose	150	50	50	50	50
Salt Mix #210050[3)]	35	35	35	35	35
Vitamin Mix#310025[4)]	10	10	10	10	10
L-Cystine	1.8	1.8	1.8	1.8	1.8
Choline Bitartrate	2.5	2.5	2.5	2.5	2.5
AS		100			
AC			100		
AF				100	
AT					100
Total	>1100	>1100	>1100	>1100	>1100

1) AIN-93 Diet
2) CONT: Control; AS: *allium sativum*; AC: *allium cepa*; AF: *allium fistulosum*; AT: *allium tuberosum*
3) AIN-93 mineral mixture (g/kg) :
Calcium carbohante anhydros 357.0, Potassium phosphate monobasic 250.0, Potassium citrate (tripotassium monohydrate) 28.0, sodium chloride 74.0, Potassium sulfate 46.6, Magnesium oxide 24.0, Ferric citrate 6.06, Zinc carbonate 1.65, Sodium meta-silicate · 9 H_2O 1.45, Manganous carbonate 0.63, Cupric carbonate 0.30, Chromium potassium sulfate · 12 H_2O 0.275, Boric acid (mg) 81.5, Sodium fluoride (mg) 63.5, Nickel carbonate (mg) 31.8, Lithium chloride (mg) 17.4, Sodium selenate anhydrous (mg) 10.25, Potassium iodate (mg) 10.0, Ammonium paramolybdate · 4 H_2O 7.95, Ammonium vanadate (mg) 6.6, Powdered sucrose 209.806
4) AIN-93 vitamin mixture (g/kg) :
Nicotinic acid 3.0, Ca pantothenate 1.6, Pyridoxine-HCl 0.7, Thiamin-HCl 0.6, Riboflavin 0.6, Folic acid 0.2, Biotin 0.02, Vitamin B-12 (cyanocobalamin) 2.5, Vitamin E (all-*rac*-α-tocopheryl acetate) 15.0, Vitamin A (all-*trans*-retinyl palmitate) 0.8, Vitamin D-3 (cholecalciferol) 0.25, Vitamin K-1 (phylloquinone) 0.075, Powdered sucrose 974.655

2. Exercise and Sample Collection

At the end of week 4, animals in each dietary group were subdivided into 3 exercise groups: before-exercise (BE); during-exercise (DE); after-exercise (AE). BE groups were sacrificed without exercise at the end of week 4. Exercised groups were exercised on a treadmill (100° incline, 0.5~0.8 km/h) for 1 hour; animals in the AE groups were allowed to recuperate for 2 hours after exercise. At the respective time points, animals were sacrificed by decapitation under light ether anesthesia. Immediately following decapitation, liver and skeletal muscle (gastrocnemius) were rapidly removed and stored at -40 ℃ until analyzed.

3. Biochemical Analysis

Pyridoxal 5'-phosphate (PLP) was measured by HPLC method,[22] 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 porus 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 perchloric acid (1 M) and allowed to sit for one hour to release PLP from protein. This mixture was then centrifuged (18,000×g, 4 ℃, 15 min) and the supernatant was removed. Fifty μl aliquot of supernatant was loaded in the sample loop and then injected onto the column. Samples for vitamin B6 analysis were prepared under yellow fluorescent lighting to prevent the photodegradation of vitamers.[23]

4. Statistical Analysis

All data were subjected to the 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

As shown in Table 2, at week 4, there were no differences between control group and *allium* vegetable diet groups except AS group in body weight. Because differences in body weight of AS animals were relatively small, the levels of PLP were assumed to have not been influenced by the differences of body weight. Table 3 shows the effect of AS diet on PLP levels in plasma, liver and muscle. There was no difference between AS animals and control animals in plasma PLP levels regardless of exercise. The liver PLP levels of AS animals were lower than those of control animals before and after exercise. The muscle PLP levels of AS animals were also lower than those of control animals before exercise but there was no difference during and after exercise. There was an interaction effect between AS diet and exercise on PLP levels of liver and muscle. Table 4 shows the effect of AC diet on PLP levels in plasma, liver and muscle. The plasma PLP levels of AC animals were higher than those of control animals before exercise but this PLP was decreased with exercise and lower than that of control animals after exercise. The liver PLP levels of AC animals were lower than those of control animals before and after exercise. The muscle PLP levels of AC animals were also lower than those of control animals before exercise but there was no difference during and after exercise. There was an interaction effect between AC diet and

Table 2. The effect of allium vegetables on body weight and feed efficiency ratio

	CONT[2)]	AS	AC	AF	AT
BW (g)	248.13 ±16.11[a,1)]	220.83 ±15.61[c]	240.56 ±9.12[ab]	249.72 ±13.98[a]	234.39 ±13.20[b]
FER	0.34 ±0.05[b]	0.38 ±0.04[a]	0.35 ±0.04[b]	0.35 ±0.04[ab]	0.33 ±0.03[b]

1) Values are mean±SEM, n=18
2) CONT: control; AS: *allium sativum*; AC: *allium cepa*; AF: *allium fistulosum*; AT: *allium tuberosum*
Within a given raw, those values with different superscripts are significantly different at p<0.05
Feed efficiency ratio=(final body weight-initial body weight)/total feed intake

Table 3. The effect of *allium sativum* diet on levels of pyridoxal 5'-phosphate (PLP)

	BE		DE		AE		Effect
	CONT[2)]	AS	CONT	AS	CONT	AS	
Plasma PLP (nmol/L)	75.74±24.19[b,1)]	65.46±37.51[b]	66.94±29.88[b]	48.97±23.24[b]	112.79±10.51[a]	110.15±38.50[a]	Ex[3)] Dt
Liver PLP (μmol/g)	3.29±0.70[a]	1.92±0.67[bc]	1.62±0.66[c]	1.77±0.18[bc]	2.36±0.38[b]	1.47±0.22[c]	Ex Dt Ex×Dt
Muscle PLP (μmol/g)	3.75±1.04[a]	0.91±0.27[b]	1.64±0.32[b]	1.25±0.38[b]	1.07±0.40[b]	1.02±0.21[b]	Ex Dt Ex×Dt

1) Values are mean±S.D, n=6
2) CONT: control; AS: allium sativum; BE: before exercise; DE: during exercise; AE: after exercise
3) Ex: exercise effect; Dt: *allium* vegetable diet effect; Ex×Dt: interaction between the effects of exercise and *allium* vegetable diet effect
Within a given row, those values with different superscripts are significantly different at p<0.05

Table 4. The effect of *allium* cepa diet on levels of pyridoxal 5'-phosphate **(PLP)**

	BE		DE		AE		Effect
	CONT[2)]	AC	CONT	AC	CONT	AC	
Plasma PLP (nmol/L)	75.74±24.19[c,1)]	196.95±46.51[a]	66.94±29.88[c]	75.69±27.52[c]	112.79±10.51[b]	51.84±31.97[c]	Ex[3)] Dt Ex×Dt
Liver PLP (μmol/g)	3.29±0.70[a]	0.71±0.03[d]	1.62±0.66[c]	0.72±0.17[d]	2.36±0.38[b]	0.27±0.08[d]	Ex Dt Ex×Dt
Muscle PLP (μmol/g)	3.75±1.04[a]	1.01±0.44[b]	1.64±0.32[b]	1.25±0.13[b]	1.07±0.40[b]	1.39±0.24[b]	Ex Dt Ex×Dt

1) Values are mean±S.D, n=6
2) CONT: control; AC: *allium* cepa; BE: before exercise; DE: during exercise; AE: after exercise
3) Ex: exercise effect; Dt: *allium* vegetable diet effect; Ex×Dt: interaction between the effects of exercise and *allium* vegetable diet effect
Within a given row, those values with different superscripts are significantly different at p<0.05

Table 5. The effect of *allium fistulosum* diet on levels of pyridoxal 5'-phosphate (PLP)

	BE		DE		AE		Effect		
	CONT[2)]	AF	CONT	AF	CONT	AF			
Plasma PLP (nmol/L)	75.74±24.19[b,1)]	76.56±20.30[b]	66.94±29.88[b]	115.81±24.15[a]	112.79±10.51[a]	95.28±39.75[ab]	Ex[3)]	-	Ex×Dt
Liver PLP (μmol/g)	3.29±0.70[a]	0.64±0.15[d]	1.62±0.66[c]	0.69±0.16[d]	2.36±0.38[b]	0.52±0.14[d]	Ex	Dt	Ex×Dt
Muscle PLP (μmol/g)	3.75±1.04[a]	0.94±0.18[a]	1.64±0.32[a]	1.01±0.29[a]	1.07±0.40[a]	0.94±0.55[a]	Ex	Dt	Ex×Dt

1) Values are mean±S.D, n=6
2) CONT: control; AF: *allium* fistulosum; BE: before exercise; DE: during exercise; AE: after exercise
3) Ex: exercise effect; Dt: *allium* vegetable diet effect; Ex×Dt: interaction between the effects of exercise and *allium* vegetable diet effect
Within a given row, those values with different superscripts are significantly different at $p<0.05$

Table 6. The effect of *allium tuberosum* diet on levels of pyridoxal 5'-phosphate (PLP)

	BE		DE		AE		Effect		
	CONT[2)]	AT	CONT	AT	CONT	AT			
Plasma PLP (nmol/L)	75.74±24.19[cd,1)]	136.48±31.16[ab]	66.94±29.88[d]	133.51±41.38[ab]	112.79±10.51[bc]	163.85±53.44[a]	Ex[3)]	Dt	-
Liver PLP (μmol/g)	3.29±0.70[a]	0.84±0.30[d]	1.62±0.66[c]	0.66±0.32[d]	2.36±0.38[b]	1.10±0.27[cd]	Ex	Dt	Ex×Dt
Muscle PLP (μmol/g)	3.75±1.04[a]	0.83±0.43[c]	1.64±0.32[bc]	1.57±0.43[bc]	1.07±0.40[bc]	1.80±0.53[b]	Ex	Dt	Ex×Dt

1) Values are mean±S.D, n=6
2) CONT: control; AT: *allium* tuberosom; BE: before exercise; DE: during exercise; AE: after exercise
3) Ex: exercise effect; Dt: *allium* vegetable diet effect; Ex×Dt: interaction between the effects of exercise and *allium* vegetable diet effect
Within a given row, those values with different superscripts are significantly different at $p<0.05$

exercise on PLP levels of plasma and tissues. Table 5 shows the effect of AF diet on PLP levels in plasma, liver and muscle. The plasma PLP levels of AF animals were higher than those of control animals during exercise but there was no difference before and after exercise. The liver PLP levels of AF animals were lower than those of control animals regardless of exercise. The muscle PLP levels of AF animals were also lower than those of control animals before exercise but there was no difference during and after exercise. There was an interaction effect between AF diet and exercise on PLP levels of plasma and tissues. Table 6 shows the effect of AT diet on PLP levels in plasma, liver and muscle. The plasma PLP levels of AT animals were higher than those of control animals regardless of exercise. The liver PLP levels of AT animals were lower than those of control animals regardless of exercise. The muscle PLP levels of AT animals were also lower than those of control animals before exercise but there was no difference during and after exercise. There was an interaction effect between AT diet and exercise on PLP levels of liver and muscle.

DISCUSSION

This study demonstrated that *allium* vegetable intake induced an alteration in the redistribution of PLP, which was most evident in rats fed AT diet with exercise. The evidence of PLP redistribution during exercise is based on the changes of PLP levels in plasma and tissues. Because exercise induces glycogenolysis and transamination of amino acids.[24,25)] it has been speculated that the increase in plasma PLP results from the mobilization of stored PLP for use by muscle or liver.[26,27)] If the exercise induced PLP rise in plasma is supplied by liver or muscle, the PLP concentration of liver or muscle should be decreased with exercise. In control rats, exercise tended to increase the PLP concentration in plasma. The results of this study were consistent with changes observed following exercise in humans[28,29)] and animals.[27)] Thus, the increased hepatic need of PLP in exercising rats would result in a release of PLP from the storage site into plasma during or after exercise.[30)] These points support the PLP redistribution hypothesis with exercise in control rats.

Compared to control animals, plasma PLP levels of

AT animals were higher than those of control animals regardless of exercise. Carbohydrate stores in muscles and liver are important for sustained energy. Meeting the energy needs helps prevent fatigue during exercise and therefore the observation that AT animals had significantly higher levels of plasma PLP than did control animals with exercise suggests AT animals utilize energy more efficiently during exercise.

In AF animals, less PLP would be synthesized in the liver and stored in muscles. However, this deprivation effect might be overcome by the increased need of PLP due to exercise in AF animals. Thus, plasma PLP levels of AF animals were not higher before exercise but higher than those of control animals with exercise. Therefore, the AF diet might also help delay fatigue and improve exercise performance by facilitating the utilization of PLP on limited fuel stores. This utilization of plasma PLP during exercise could also be a factor that limits performance capacity in prolonged exercise. Thus, it is suggested that AF or AT diet has a potential to increase plasma PLP and to utilize energy more efficiently during exercise.

Because the liver and muscle PLP levels of AS and AC animals were lower than those of control animals before exercise, less PLP would be also synthesized in the liver and stored in muscles in AS and AC animals. This deprivation effect might be greater than any PLP increase due to exercise. These results are consistent with the report that the vitamin B6 status and vitamin B6 intak e of high performance athletes were lower than those of untrained individuals.[31] Thus, compared to control ani mals, it is assumed that plasma PLP levels of AC animals were lower than those of control animals with exercise or plasma PLP levels of AS animals tended to be lower than those of control animals with exercise, although this difference was not statistically significant. Moreover, the increased plasma need for PLP with exercise in AS and AC animals would result in even more decrease in the PLP levels of liver and muscle with exercise. Although some *allium* vegetables are considered as blood lipid prof ile altering agents,[32-35] AS and AC did not have the poten tial to utilize PLP more efficiently.

In summary, this study shows that the changes of PLP concentrations in plasma, liver and muscle induced by exercise are affected by *allium* vegetable diet and it is most evident in rats fed AT diet with exercise. Thus, it is suggested that AF or AT diet has a potential to increase plasma PLP and to utilize energy more efficiently during exercise and AS and AC do not.

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