Effect of Exercise on the Metabolism of Vitamin B6 and Some PLP-dependent Enzymes in Young Rats Fed a Restricted Vitamin B6 Diet

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Summary The effect of exercise on vitamin B6 metabolism and PLP-dependent enzymes was studied in rats fed a diet with or without vitamin B6. Metabolism of some amino acids (citrulline, arginine, ornithine and threonine) inhibited in the B6-deficient rats was normalized during exercise. Exercise was also effective in storing vitamin B6 in the body by lowering excretion of vitamin B6, when intake of vitamin B6 was restricted. Aspartatae aminotransferase activity was higher in the red portion of the gastrocnemius muscle than that of the white one, whereas glycogen phosphorylase activity was vice versa and furthermore glycogen content in the white portion was very low in the vitamin B6-deficient rat. From the data obtained, it has been suggested that the red and white portions of the gastrocnemius muscle seemed to be more important in metabolizing amino acids and hydrolyze glycogen, respectively.

Key Words vitamin B6, exercise, amino acid metabolism, glycogenolysis, gastrocnemius muscle

It is well known that glycogen and amino acids are mainly metabolized by the PLP-dependent enzymes such as glycogen phosphorylase and aminotransferases. It has been known that, by exercise, the synthesis and the degradation of muscle protein increase, and glycogenolysis is stimulated. Hadi-Saad et al. (1) have suggested that prolonged exercise affects the metabolism of vitamin B6, but does not increase vitamin B6 requirements in growing rats. They observed that physical exertion resulted in an increase in urinary 4-pyridoxic acid (4-PA) excretion and some alterations in the forms of B6 vitamers contained in the tissues in a state of physical exertion, but no alteration in the quantity of B6. However, Dreon and Butterfield (2) reported that 4-PA excretion was decreased in their male subjects by running. In fact, to date, there is no clear documentation whether exercise increases or decreases the loss of vitamin B6 from the system. Therefore, further research with a focus on vitamin B6 and exercise is necessary in order to determine whether or not vitamin B6 requirements are increased by exercise (3, 4).

Amino acid metabolism is an interesting subject in relation to a study of exercise and vitamin B6. Prolonged exercise can elicit a reduction in plasma glutamine concentrations and an increase in the plasma concentration ratio of free tryptophan to branched-chain amino acids (5–7).

The purpose of this study was to discover whether or not alterations in vitamin B6 metabolism and pyridoxal 5'-phosphate (PLP)-dependent enzyme activity, including glycogenolysis and amino acid metabolism, occur during exercise.

MATERIALS AND METHODS

Animals. Two series of experiments were performed. In experiment 1, 30 male weanling (3-wk-old) Wistar rats were divided into 4 groups. Groups 1 (n=10) and 2 (n=5) were fed a 20% casein diet containing 1.5 mg/kg pyridoxine hydrochloride, and groups 3 (n=10) and 4 (n=5) were fed a 20% casein diet without supplemented pyridoxine for 5 wk. In experiment 2, 15 rats were divided into two groups (n=8, group 1; n=7, group 2) and the rats were fed a diet similar to that of groups 1 and 2 in experiment 1. All animals had free access to tap water, and body weight and food intake were estimated daily. Other dietary components have been described previously (8).

Exercise program. Rats were subjected to a swimming program. Treatment consisted of swimming up to 30 min every other day (groups 1 and 3 in experiment 1) for 4 wk and 1 h every day (group 1 in experiment 2) for 3 wk, after a week-long adaptation period. Groups 2 and 4 (experiment 1), and group 2 (experiment 2) did not exercise and served as controls.

Chemicals. All reagents used were of the highest grade available and were purchased from Wako Pure Chemicals Industries (Osaka, Japan), Boehringer (Mannheim, Germany), Sigma Chemicals Co. (St. Louis, MO, USA), or Oriental Yeast Co. (Osaka, Japan). All dietary materials were purchased from Oriental Yeast Co.

Sampling and preparation. Blood was collected into heparinized tubes, centrifuged at 2,000 rpm for 10 min; the resultant precipitate was washed twice with equal
volumes of saline and suspended in 2 volumes of water. The suspension was then subjected to sonic oscillation and the supernatant, centrifuged at 10,000×g for 20 min, was used to measure aspartate aminotransferase (AST) [EC 2.6.1.1] activity and hemoglobin. The plasma fraction was used for the quantification of B$_6$ vitamers and amino acids. For the determination of B$_6$ vitamers, 0.5 mL of plasma was diluted to 1 mL with water, and 27 µL of 9 N-HClO$_4$ was added. Using voltex, samples were mixed for 1 min and centrifuged at 10,000×g for 5 min, and the resultant, supernatant was collected. For the analysis of amino acids, 1 mL of the plasma fraction was mixed with 2 mL of 10% TCA, and the supernatant was centrifuged at 10,000×g for 10 min. A urine sample (24 h) from each rat on the final day of experiment 2 was collected in a flask containing 1 mL 1 N-HCl, and the supernatant, centrifuged at 10,000×g for 10 min, was used to analyze 4-PA and urea. Rat tissue was removed, divided into 2 parts, and was frozen until used. Gastrocnemius muscle tissue was excised and separated visually into red and white portions. Preparation of tissue samples used for analyses of vitamin B$_6$ levels and enzyme activity was substantially performed as described previously (9). Briefly, tissues were homogenized with 9 volumes of 1 N-HClO$_4$ and centrifuged, and the supernatant was used for the determination of the vitamers. Tissues were homogenized with phosphate buffer and PIPES buffer for the analyses of AST and glycogen phosphorylase [EC 2.4.1.1.] activity, respectively. The homogenates were centrifuged at 10,000×g for 20 min and the supernatants were used for the analyses. Anti-rat cytosolic AST (cAST) antisera was prepared in rabbits, as described previously (10).

Methods. B$_6$ vitamers were determined using high performance liquid chromatography (HPLC) according to methods originally described by Edwards et al. (11) and Takashi and Shibuya (12). A Shimadzu RF-535 fluorescence monitor was used to detect the fluorescence of vitamin B$_6$ (ex. 325 nm, em. 420 nm). Glycogen phosphorylase activity was determined by analyzing inorganic phosphate levels during the formation of glycogen in the presence or absence of 1 mM AMP (13). The AST activity was determined in the presence or absence of 10-4M PLP by measuring the rate of NADH oxidation in the presence of malate dehydrogenase, as described by Karmen (14). cAST activity (in the liver, muscle, and kidney) was determined by subtracting the mitochondrial AST activity from the total AST activity, in which the mitochondrial activity was obtained using anti-cAST antiserum (9). AST activity in the erythrocytes was followed by preincubation with lactate dehydrogenase to remove endogenous pyruvate prior to starting the AST reaction (15). Hemoglobin was determined using a Wako Hemoglobin B kit. The glycogen content was obtained by subtracting the glucose content that had been obtained in the sample without acid hydrolysis (13). Amino acids were analyzed using HPLC (Hitachi 835) and the detection was carried out with ninhydrin reagent at OD$_{570}$.

### RESULTS

#### Growth of rats

The effect of exercise on growth of rats was not observed in experiment 1; however, in experiment 2 weight gain of the exercised group was significantly lower than that of the not-exercised group (Table 1). This effect of exercise was observed within a week after feeding and then continued until final stage of the experiment.

**Experiment 1**

**Vitamin B$_6$ content in the tissues.** As shown in Table 2, vitamin B$_6$ content in the liver and kidney from B$_6$-deficient rats decreased to 2/3 of that of the B$_6$-supplemented controls. In the B$_6$-deficient rats, the effects of exercise were observed at the level of pyridoxamine 5'-phosphate (PMP), which increased in liver and decreased in kidney, as compared with that of the not-exercised control group. In the gastrocnemius muscle of vitamin B$_6$-supplemented rats, the PMP level increased with exercise, suggesting amino acid metabolism in the muscle may be promoted by exercise. Thus, in all of the tissues tested, changes in vitamin B$_6$ content brought about by exercise were observed only in the case of PMP levels. The vitamin B$_6$ content in the liver and kidney was more resistant to B$_6$-deficiency, while that in the skeletal muscle was very sensitive to the deficiency.

**AST and glycogen phosphorylase activity and glycogen content in the gastrocnemius muscle (Table 3).** AST activity was determined in the presence and absence of PLP. When PLP was present, the level of the AST activity of the B$_6$-deficient rats had completely recovered to that of the B$_6$-supplemented rats. No effect of exercise was observed under the conditions employed. Some difference between the red and the white muscle tissue was observed in all groups, showing higher activity in the red muscle than in the white muscle tissue. Glycogen phosphorylase activity was analyzed in the

| Experiment | Group          | Initial body weight (g) | Final body weight (g) |
|------------|----------------|-------------------------|-----------------------|
| 1          | Exercised+B$_6$ | 49.4±2.8$^{a}$          | 271.4±17.8$^{a}$      |
|            | Not-exercised+B$_6$ | 48.2±3.7$^{a}$          | 281.0±16.3$^{a}$      |
|            | Exercised−B$_6$  | 49.6±2.4$^{a}$          | 177.8±15.1$^{b}$      |
|            | Not-exercised−B$_6$ | 50.3±4.6$^{a}$          | 194.0±20.4$^{b}$      |

Values are means±SD. Data were analyzed by Scheffe's multiple test. Within a column and same series of experiment, values are not significantly different if a superscript contains a common letter ($p<0.05$).

**Table 1. Growth of rats in experiments 1 and 2.**

| Experiment | Group          | Initial body weight (g) | Final body weight (g) |
|------------|----------------|-------------------------|-----------------------|
| 1          | Exercised+B$_6$ | 38.6±1.8$^a$            | 199.1±14.0$^a$       |
|            | Not-exercised+B$_6$ | 38.4±1.6$^b$            | 219.7±13.6$^b$       |

Values are means±SD. Data were analyzed using either Student’s $t$-test or Scheffe’s multiple test to determine which means were significantly different from each other.
Table 2. Vitamin B6 determination in the tissue of exercised rats.

| Conditions      | Exercised + B6 | Not exercised + B6 | Exercised − B6 | Not exercised − B6 |
|-----------------|----------------|-------------------|----------------|-------------------|
| Plasma (nmol/L) |                |                   |                |                   |
| PLP             | 0.078±0.016    | 0.087±0.013       | trace          | trace             |
| PL              | 0.045±0.01     | 0.049±0.011       | trace          | trace             |
| Total           | 0.124±0.023    | 0.136±0.023       | trace          | trace             |
| Liver (nmol/g)  |                |                   |                |                   |
| PMP             | 22.44±1.23     | 22.38±0.88        | 17.4±1.12      | 17.4±1.06         |
| PLP             | 13.30±0.79     | 12.70±1.33        | 5.69±0.69      | 5.16±0.52         |
| Total           | 35.74±1.34     | 35.08±1.50        | 24.42±1.37     | 22.60±1.21        |
| Kidney (nmol/g) |                |                   |                |                   |
| PMP             | 56.24±5.27     | 54.89±2.38        | 34.54±2.49     | 39.41±4.09        |
| PLP             | 5.39±0.74      | 5.92±0.38         | 1.90±0.34      | 2.24±0.36         |
| Total           | 61.63±5.69     | 60.81±2.30        | 36.44±2.53     | 41.65±4.38        |
| Red muscle (nmol/g) |            |                   |                |                   |
| PMP             | 8.28±1.03      | 7.00±0.41         | 1.80±0.29      | 1.68±0.32         |
| PLP             | 15.23±2.40     | 14.54±1.07        | 4.29±0.36      | 4.73±0.86         |
| Total           | 23.51±3.02     | 21.54±1.38        | 6.09±0.45      | 6.41±1.14         |
| White muscle (nmol/g) |        |                   |                |                   |
| PMP             | 3.25±0.46      | 3.27±0.52         | 0.49±0.21      | 0.36±0.18         |
| PLP             | 19.22±0.54     | 18.56±1.17        | 4.60±0.25      | 4.85±0.66         |
| Total           | 22.47±0.78     | 21.83±1.54        | 5.09±0.38      | 5.21±0.73         |

Values are means±SD.
Data were analyzed by Scheffe’s multiple test. Within a row, values are not significantly different if and only if superscripts (a, b, c) contain at least one common letter (p<0.05). Data were also compared between red and white muscle tissues in each dietary group; the results of this analysis are indicated as superscripts (1, 2, 3).

Table 3. Aspartate aminotransferase and glycogen phosphorylase in the gastrocnemius muscle of rats trained in experiment 1.

| Conditions                | Exercised + B6 | Not exercised + B6 | Exercised − B6 | Not exercised − B6 |
|---------------------------|----------------|-------------------|----------------|-------------------|
| Aspartate aminotransferase (µkat/100 g) |                |                   |                |                   |
| Red muscle + PLP          | 44.37±7.90     | 36.52±7.69        | 40.03±4.63     | 35.58±7.59        |
| − PLP                     | 36.70±8.89     | 25.88±2.70        | 7.26±1.63      | 7.26±2.53         |
| White muscle + PLP        | 23.59±5.04     | 18.83±5.54        | 18.63±3.61     | 14.93±5.80        |
| − PLP                     | 14.85±3.86     | 9.82±2.40         | 1.90±0.67      | 1.52±0.68         |
| Glycogen phosphorylase (µkat/100 g) |                |                   |                |                   |
| Red muscle + AMP          | 52.00±6.52     | 56.11±10.66       | 15.56±4.49     | 14.06±8.84        |
| − AMP                     | 42.58±5.19     | 44.49±7.04        | 14.44±6.11     | 9.22±7.58         |
| White muscle + AMP        | 288.9±48.2     | 279.8±41.2        | 41.81±8.34     | 48.90±9.50        |
| − AMP                     | 205.0±53.8     | 256.0±36.1        | 29.91±6.76     | 38.92±7.16        |

Values are means±SD.
Data were analyzed by Scheffe’s multiple test. Within a row, values are not significantly different if superscripts (a, b, c) contain a common letter, and asterisks are significantly different, when compared with the corresponding value from −PLP (p<0.05).

presence (total) and absence (active) of AMP. This enzyme activity was much higher in the white than in the red muscle, although no effect of exercise was observed. Thus, white muscle tissue appeared to contribute largely to glycogen degradation. In contrast to the case of AST activity, glycogen phosphorylase activity was very sensitive to B<sub>6</sub>-deficiency; the activity in the B<sub>6</sub>-deficient rats was only 15% and 25% of the control in the white and red muscle tissues, respectively. However, glycogen phosphorylase activity was still higher in the white muscle tissue than in the red muscle. The glycogen content in the red muscle tissue was much higher in the deficient rats than in that of the controls (Table 4).

Amino acids in plasma. It is well known that amino acid metabolism is impaired in cases of B<sub>6</sub>-deficiency (16). In this study, certain amino acids increased and others decreased in the plasma of the B<sub>6</sub>-deficient rats, as compared with that of the controls (Table 5). No effect of exercise was observed in the B<sub>6</sub>-supplemented groups; however, the amounts of some amino acids, such as citrulline, arginine, ornithine, and threonine,
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Table 4. Glycogen content in the liver and gastrocnemius muscle of rats trained in experiment 1.

|          | Exercised + B6 | Not exercised + B6 | Exercised − B6 | Not exercised − B6 |
|----------|----------------|--------------------|----------------|--------------------|
| Glycogen (mg/g) |                |                    |                |                    |
| Red muscle | 3.455 ± 0.474^a | 2.568 ± 0.404^a | 6.355 ± 2.053^bc | 4.815 ± 2.113^bc |
| White muscle | 2.740 ± 1.193^a | 2.075 ± 0.455^a | 0.963 ± 0.266^b  | 0.978 ± 0.556^b  |
| Liver     | 59.97 ± 8.14^a  | 59.93 ± 4.37^bc   | 80.12 ± 7.66^bc | 79.65 ± 3.21^bc   |

Values are means ± SD.

Data were analyzed by Scheffe’s multiple test. Within a row, values are not significantly different if and only if superscripts (a, b, c) contain at least one common letter (p<0.05).

Table 5. Amino acid content in plasma in experiment 1.

| Amino acid (μmol/L) | Exercised + B6 | Not exercised + B6 | Exercised − B6 | Not exercised − B6 |
|---------------------|----------------|--------------------|----------------|--------------------|
| Asp + Asn           | 0.549 ± 0.565^a | 0.266 ± 0.600^a | 0.383 ± 0.127^a | 0.335 ± 0.058^a |
| Thr                 | 12.39 ± 1.52^b,c | 11.87 ± 1.20^a   | 12.70 ± 1.81^b  | 14.87 ± 1.81^b   |
| Ser                 | 5.198 ± 0.584^a | 5.111 ± 0.699^a  | 4.517 ± 0.565^a | 4.869 ± 0.685^a  |
| Glu + Gln           | 2.857 ± 0.584^a | 3.166 ± 0.445^a  | 3.149 ± 0.810^a | 3.033 ± 0.665^a  |
| Gly                 | 3.986 ± 0.713^a | 3.919 ± 0.639^a  | 8.378 ± 1.549^b | 7.530 ± 1.65^b  |
| Ala                 | 11.88 ± 1.57^a  | 10.92 ± 0.96^a   | 8.27 ± 1.22^b   | 7.51 ± 1.14^b   |
| Cit                 | 2.316 ± 0.490^a | 2.428 ± 0.343^a  | 1.795 ± 0.802^c | 0.825 ± 0.109^b |
| Val                 | 4.679 ± 0.401^a | 4.617 ± 0.686^a  | 6.082 ± 1.185^b | 5.162 ± 0.609^a |
| Cys                 | 0.346 ± 0.064^a | 0.403 ± 0.059^a  | 0.237 ± 0.103^b | 0.139 ± 0.031^b |
| Met                 | 1.110 ± 0.138^a | 1.055 ± 0.250^a  | 1.448 ± 0.234^b | 1.449 ± 0.169^b |
| Cysthi              | 0.037 ± 0.010^a | 0.040 ± 0.005^a  | 0.417 ± 0.115^b | 0.382 ± 0.124^a |
| Ile                 | 2.076 ± 0.135^a | 2.141 ± 0.323^a  | 2.465 ± 0.514^a | 2.198 ± 0.190^b |
| Leu                 | 2.968 ± 0.257^a | 3.220 ± 0.578^b  | 4.190 ± 1.105^b | 4.284 ± 0.466^b |
| Tyr                 | 2.380 ± 0.295^a | 2.425 ± 0.673^a  | 2.482 ± 0.491^a | 2.057 ± 0.387^a |
| Phe                 | 1.025 ± 0.233^a | 1.266 ± 0.101^a  | 1.028 ± 0.207^a | 0.961 ± 0.094^a |
| Orn                 | 0.968 ± 0.075^a | 1.050 ± 0.064^a  | 1.448 ± 0.153^b | 2.434 ± 0.784^a |
| Lys                 | 12.33 ± 0.98^a  | 12.28 ± 1.52^a   | 11.30 ± 1.13^a  | 10.72 ± 0.96^a  |
| His                 | 1.263 ± 0.142^a | 1.270 ± 0.137^a  | 1.451 ± 0.360^b | 1.435 ± 0.126^ab |
| Arg                 | 2.526 ± 0.231^a | 2.782 ± 0.427^a  | 2.229 ± 0.360^a | 1.425 ± 0.526^a |
| Pro                 | 2.318 ± 0.322^a | 2.194 ± 0.435^a  | 3.123 ± 1.381^a | 2.282 ± 0.842^a |

Values are means ± SD.

Data were analyzed by Scheffe’s multiple test. Within a row, values are not significantly different if and only if superscripts contain at least one common letter (p<0.05).

changed in the plasma of the B6-deficient rats with exercise. The levels of these amino acids showed a tendency to return to the normal level with exercise.

Experiment 2

Vitamin B6 content and excretion. Vitamin B6 content in the tissues and the urinary 4-PA of rats trained in experiment 2 are shown in Table 6. Urinary 4-PA is known to be the main excreted form of B6 vitamers. PMP in the liver and in muscle tissue (both red and white) increased in the exercised group. The results obtained in the liver were similar to those of the B6-deficient group in experiment 1. On the other hand, the exercised rats excreted only half the amount of 4-PA, as compared with that excreted from the not-exercised control rat.

Aspartate aminotransferase isozyme and glycogen phosphorylase activities. As the occurrence of isozyme for AST is well known, the isozyme activities were separately determined in the various tissues (Table 7). Increases in the cytosolic activity of isozyme in all of the studied tissues, except for the liver, of exercised rats were observed, reflecting an elevated metabolism of amino acid in these tissues. In the gastrocnemius muscle, mitochondrial enzyme also increased in the group of exercised rats. A decrease in glycogen phosphorylase activity was observed in the white muscle tissue from the exercised rats (Table 8).

DISCUSSION

We expected that vitamin B6 requirements might be elevated during periods of exercise because PLP-dependent enzymes, such as glycogen phosphorylase and aminotransferase, play an important role in supplying energy from carbohydrate and amino acid sources. Young rats were fed on a 20% protein diet containing 0 or 1.5 mg/kg of vitamin B6; the latter amount was considered to be the lowest limit to maintain normal status regarding amino acid and glycogen metabolism (17). Exercise training was conducted moderately in experiment 1 and more severely in experiment 2. As the gas-
Table 6. Vitamin B₆ metabolism in the rats trained in experiment 2.

|                      | Exercised     | Not exercised |
|----------------------|---------------|---------------|
| **Plasma (nmol/L)**  |               |               |
| PLP                  | 0.076±0.060   | 0.051±0.038   |
| PL                   | 0.095±0.040   | 0.076±0.027   |
| Total                | 0.170±0.097   | 0.127±0.065   |
| **Liver (nmol/g)**   |               |               |
| PMP                  | 19.22±1.19*   | 17.36±1.55    |
| PLP                  | 10.69±1.13*   | 13.37±2.07    |
| Total                | 29.91±2.03    | 30.73±3.56    |
| **Kidney (nmol/g)**  |               |               |
| PMP                  | 73.75±3.36    | 68.06±6.56    |
| PLP                  | 5.51±0.58     | 4.91±0.49     |
| Total                | 79.26±3.65*   | 72.97±6.78    |
| **Muscle (red)**     |               |               |
| PMP                  | 9.25±0.98*    | 7.14±0.54     |
| PLP                  | 15.11±1.99    | 14.44±2.19    |
| Total                | 24.36±2.58    | 21.58±2.47    |
| **Muscle (white)**   |               |               |
| PMP                  | 3.77±0.59*    | 2.80±0.37     |
| PLP                  | 17.57±1.80    | 17.23±2.34    |
| Total                | 21.34±2.17    | 20.03±2.69    |
| **Urine (nmol/d)**   |               |               |
| PLA                  | 13.85±2.69*   | 28.84±10.50   |

Values are means±SD.
* Significantly different compared with the corresponding not-exercised rats.

Table 7. Aspartate aminotransferase activity in the various tissues of rats trained in experiment 2.

|                      | Mitochondria                      | Cytosol                        |
|----------------------|-----------------------------------|--------------------------------|
|                      | Exercised                        | Not exercised                  | Exercised                        | Not exercised                  |
| Erythrocyte (μkat/g Hb) + PLP | 29.3±3.2 | 26.3±3.8 | 63.2±9.9* | 46.6±9.0 |
| − PLP                | 26.6±3.0 | 25.3±4.0 | 52.6±8.1* | 37.7±7.6 |
| Liver (μkat/100 g)   | + PLP                | 15.6±0.7 | 15.4±1.1 | 3.9±0.7  | 4.0±0.9 |
| − PLP                | 13.7±0.8 | 13.5±1.0 | 3.9±0.3* | 3.2±0.3 |
| Kidney (μkat/100 g)  | + PLP                | 27.7±3.1* | 22.4±2.7 | 2.5±0.4* | 1.9±0.3 |
| − PLP                | 20.3±3.5 | 18.1±2.2 | 13.6±2.1* | 10.8±1.2 |
| Gastrocnemius Red (μkat/100 g) | + PLP | 11.9±1.0* | 9.7±0.8 | 6.0±1.3 | 4.7±0.9 |
| − PLP                | 8.6±0.8 | 7.1±0.9 | 3.4±0.8 | 2.8±0.4 |

Values are means±SD. * Significantly different compared with the corresponding value from not-exercised rats.

Table 8. Glycogen phosphorylase activity in the gastrocnemius muscle of rats trained in experiment 2.

|                      | Exercised (μkat/100 g) | Not exercised (μkat/100 g) |
|----------------------|------------------------|---------------------------|
|                      | + AMP                  | − AMP                     | + AMP                  | − AMP                     |
| Red muscle           | 50.1±3.6               | 45.1±3.8                 | 51.8±5.7               | 44.3±4.9                 |
| White muscle         | 289.1±51.1             | 188.5±51.3*              | 280.0±40.0             | 235.1±38.2               |

Values are means±SD.
* Significantly different compared with the corresponding value from not-exercised rats.

trocnemius muscle contains both red and white portions, each was separately analyzed for B₆ content and for PLP-dependent enzyme activity. Glycogen phosphorylase activity was observed largely in the white muscle and was severely depressed in the B₆-deficient rats; however, levels were still higher than those in the red muscle (Table 3). The decreased level of glycogen phosphorylase activity in the white muscle of B₆-deficient rats appeared nonetheless sufficient for metabolizing glycogen, because the glycogen content in the white muscle tissue of the deficient rats was lower than in that of the controls (Table 4). In experiment 2, rats were subjected to a more severe exercise than those in experiment 1. In the rats of experiment 2, aspartate aminotransferase activities were elevated by exercise, except for those in the liver and white muscle tissue. This finding suggests that this enzyme plays an important role in the supplement of energy from amino acids by exercise (Table 7). Vitamin B₆ metabolism was altered in this experiment, i.e., more B₆ was retained in the body and less B₆ was excreted into the urine during the period of exercise (Table 6). It will be suggested that exercise may necessitate the intake of more B₆; however it also may allow B₆-deficient rats to achieve a normal state by reducing B₆ consumption. Vitamin B₆ interconversion also resulted in some changes with exercise. Throughout the two series of experiments, PMP was usually elevated in the exercised rats, even in the cases in which total B₆ content was not altered. It has been reported that amino acid metabolism is
inhibited in B<sub>6</sub>-deficient animals (16). In experiment 1, we analyzed amino acids in the plasma from all 4 groups; changes in the levels of most amino acids were observed in the plasma from the B<sub>6</sub>-deficient groups (Table 5). Levels of citrulline and arginine were elevated, whereas those of threonine and ornithine decreased in the exercised rats. However, those levels normalized during the period of exercise. An increase of ornithine and threonine in the plasma of B<sub>6</sub>-deficient rats is due to a decrease in ornithine aminotransferase and threonine dehydrase, which are both PLP-dependent enzymes. Although we determined ornithine aminotransferase activities in liver and kidney, no significant change of the activity was observed between exercised and not-exercised groups (data not shown). Virk et al. (18) reported the vitamin B<sub>6</sub> supplementation can alter plasma amino acid concentrations during exhaustive endurance exercise, without affecting endurance. Considering our data together with those of previous studies, it is possible that exercise necessitates the intake of more vitamin B<sub>6</sub> than no exercise. However, it should be noted that exercise is good for the maintenance of the health of an animal, even for that of a malnourished animal. Although we do not know if, or how, vitamin B<sub>6</sub> metabolism is favorably regulated during exercise, this remains a very interesting subject for future research.

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