Exercise and a High Fat Diet Synergistically Increase the Pantothenic Acid Requirement in Rats

Kei TAKAHASHI1, Tsutomu FUKUWATARI2 and Katsumi SHIBATA2

1Department of Health and Nutrition, Faculty of Health and Human Life, Nagoya Bunri University, Inazawa, Aichi 492–8520, Japan
2Department of Nutrition, School of Human Cultures, The University of Shiga Prefecture, Hikone, Shiga 522–8533, Japan

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Summary It is thought that both exercise and dietary composition increase the utilization of, and thus the requirement for, certain water-soluble vitamins. However, there have been no studies evaluating the combined impacts of exercise and dietary composition on vitamin utilization. In this experiment, rats were fed a pantothenic acid (PaA)-restricted (0.004 g PaA-Ca/kg diet) diet containing 5% (ordinary amount of dietary fat) or 20% fat (high fat), and were forced to swim until exhaustion every other day for 22 d. PaA status was assessed by urinary excretion, which reflects body stores of water-soluble vitamins. The urinary excretion of PaA in rats fed a 5% fat diet was not affected by swimming (5% fat + non-swimming vs. 5% fat + swim; p > 0.05). Excretion of PaA was decreased by the high-fat diet (5% fat + non-swim vs. 20% fat + non-swim; p < 0.05) and synergistically decreased by exercise (20% fat + non-swim vs. 20% fat + swim; p < 0.05). There was a significant interaction between exercise and a high-fat diet. Plasma PaA concentrations showed changes similar to those seen for urinary excretion. The experiment was then repeated using rats fed a PaA-sufficient (0.016 g PaA-Ca/kg diet) diet, and PaA excretion was again synergistically decreased by the combination of exercise and a high-fat diet (p < 0.05). These results suggest that the combination of exercise and a high-fat diet synergistically increases the requirement for PaA.

Key Words exercise, water-soluble vitamins, fat, urine, rat

B-group vitamins are necessary for several important physiological processes, including energy production, hemoglobin synthesis, immune function, and growth and repair of muscle tissue. As a person becomes more physically active, it is logical to assume that their requirement for energy and protein will increase, as will their requirement for B-vitamins. However, there are many reports of athletes with poor or marginal nutritional status for several B-group vitamins (1–4), although these studies did not compare the vitamin status of the athletes to that of sedentary individuals. Other studies have compared the performance effects of a placebo versus a vitamin supplement (5, 6); but the focus of these studies was on improvement in exercise performance, rather than on meeting the changing vitamin requirements of athletes. We have previously demonstrated an exercise-induced increase in the vitamin B1 requirement of rats (7). However, few studies have examined whether vitamin requirements are affected by the combination of exercise and the availability of specific dietary nutrients. Manore et al. (8) reported that the plasma concentration of vitamin B6, which vitamin was involved in protein metabolism, decreased with exercise regardless of level of carbohydrate in the diet.

Pantothenic acid (PaA) is a B-group vitamin important for lipid metabolism; it is essential for the synthesis of coenzyme A (CoA) (9, 10). Although there have been no studies demonstrating an exercise-associated increase in the PaA requirement, many studies have shown that fat metabolism, which is closely linked to PaA status, is increased by exercise (11, 12). We have previously reported that a high-fat diet increases the requirement for PaA (13). Therefore, in this study, to test the hypothesis that specific vitamin requirements are synergistically increased by exercise combined with a high-fat diet, we assessed the effects of swimming exercise and high dietary fat on the PaA status of rats that were fed PaA-restricted and PaA-sufficient diets.

MATERIALS AND METHODS

Chemicals. Vitamin-free milk casein, sucrose, and L-methionine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Corn oil was purchased from Ajinomoto Co., Inc. (Tokyo, Japan). Gelatinized cornstarch, a mineral mixture (AIN-93M) (14), and the vitamin mixture (AIN-93VX, PaA free) (14) were obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan).

Calcium pantothenate (PaA-Ca, C18H27N2O10-Ca = 476.54) was purchased from Wako Pure Chemical Industries. All other chemicals used were of the highest purity available from commercial sources.

Experiment 1: Animals and experimental groups. The
care and treatment of experimental animals conformed to the University of Shiga Prefecture guidelines for the ethical treatment of laboratory animals. The animal room was maintained at approximately 22°C and 60% humidity with a 12-h light (06:00–18:00)/12-h dark (18:00–06:00) cycle. Body weight and feed intake were measured daily at approximately 09:00, and the feed and water were changed daily.

Twenty just-weaned male Wistar rats (3 wk old) were purchased from CLEA Japan, Inc. (Tokyo, Japan) and immediately divided into two groups (n = 10/group). The rats were fed PaA-restricted (0.004 g PaA-Ca/kg diet) diets containing either 5% or 20% fat (Table 1), and were housed in individual rat metabolism cages (CL-0301, CLEA Japan). After 15 d of habituation to their diets and housing, all 20 rats were forced to swim in order to familiarize them with the swimming procedure. The rats in each dietary group were then assigned randomly to swimming or non-swimming groups, resulting in a total of four experimental groups: 5% fat diet + non-swimming (5%NS), 5% fat diet + swimming (5%S), 20% fat diet + non-swimming (20%NS), and 20% fat diet + swimming (20%S) group.

Swimming exercise. Rats in the swimming groups were forced to swim until exhaustion every other day for 22 d. Swimming was performed in a running-water pool (890 × 435 × 444 mm) filled to a depth of 350 mm with the water maintained at ~35°C. The circulating water current was created with a pump. All rats swam with a weight equal to 3% of their body weight tied to the base of their tail. They were judged to be “exhausted” if they could not come to the surface of the water to breathe for >7 s (7).

Sample collection. On the last day of the experiment (day 37), 24-h urine samples (from 09:00 on day 36 to 09:00 on day 37) were collected in amber bottles containing 1 mL of 1 mol/L HCl, and stored at −25°C for later measurement of the water-soluble vitamin content of the urine. Rats were killed by decapitation at 09:00 after the urine sample collection, and a 10-μL sample of blood was taken from the carotid artery to measure plasma PaA concentration. The entire liver, a femoral muscle sample, the heart drained of blood, and both adrenal glands were then immediately collected. The blood and tissue samples were prepared for the PaA assay immediately after collection and then stored at −25°C until analysis.

Biochemical analyses. The PaA levels in the samples collected were measured by a micro-bioassay using a PaA auxotrophic strain of Lactobacillus plantarum, ATCC 8014, as described previously (15). Urine samples were used directly, while blood, liver, muscle, heart and adrenal glands were first prepared for the total PaA assay. The tissue samples were incubated in a plastic-wrapped container at 37°C for 6 h to release free PaA from such bound PaA as CoA and acetyl-CoA by autolysis. The samples were then homogenized in 50 mmol/L of KH₂PO₄–K₂HPO₄ buffer (pH 7.0) at 1 : 10 (w/v), heated at 100°C for 5 min, cooled for 5 min, and centrifuged for 5 min at 20,000 × g. The supernatant was used as the assay sample. The plasma was added to 50 mmol/L of KH₂PO₄–K₂HPO₄ buffer (pH 7.0) at 1 : 10 (w/v), and treated in the same manner.

Experiment 2. After Experiment 1 was completed, to evaluate the effect of the same exercise and high fat diet combination on rats fed sufficient PaA, we repeated the experiment using the same methods but increased the amount of PaA in the diet from 0.004 g PaA-Ca/kg diet to 0.016 g PaA-Ca/kg diet (Table 1). With the exception of dietary PaA content, all aspects of Experiment 2 were the same as in Experiment 1.

Table 1. Composition of the diets.

|                     | PaA-restricted | PaA-sufficient |
|---------------------|----------------|----------------|
|                     | 5% fat diet    | 20% fat diet   | 5% fat diet    | 20% fat diet   |
| Vitamin-free milk casein | 200            | 200            | 200            | 200            |
| l-Methionine        | 2.0            | 2.0            | 2.0            | 2.0            |
| Gelatinized cornstarch | 469            | 369            | 469            | 369            |
| Sucrose             | 234            | 184            | 234            | 184            |
| Corn oil            | 50             | 200            | 50             | 200            |
| Mineral mixture     | 35             | 35             | 35             | 35             |
| (AIN-93-G-MX)¹      |                |                |                |                |
| PaA-free vitamin mixture | 10             | 10             | 10             | 10             |
| (AIN-93-VX)¹        |                |                |                |                |
| Calcium pantothenate | 0.004          | 0.004          | 0.016²         | 0.016²         |

¹ Ref. 14.
² Amount based on the vitamin composition of AIN-93-VX.

Each value is expressed as g/kg of diet.
Exercise and Fatty Diet Increase Pantothenic Acid Requirement

Exercise and fatty diet increase pantothenic acid requirement. In the experiment, the effects of each variable (swimming or high-fat diet) were evaluated independently by one-way ANOVA followed by Tukey’s multiple-comparison tests. In the experiment, the effects of PaA amount in diet were evaluated by Student’s t-test with corresponding values in the PaA-restricted diet (Experiment 1). Differences were considered to be significant if p < 0.05.

RESULTS

Body weight and feed intake in rats fed a PaA-restricted diet

Table 2 shows the body weights on the initial, 15th, and final (37th) day of the experiment, and the body weight gain, total feed and energy intakes, and feed and energy efficiency ratios in rats with restricted PaA intake. The interaction between swimming and a high-fat diet was not significant for any values. There were no significant between-group differences in body weight on day 1 or 15. Final body weight and total weight gain over the study period were significantly lower in the 20%S group than in any of the other groups. The 5%S and 20%NS groups gained significantly less weight than the 5%NS group. Feed intake was significantly lower in the rats fed the 20% fat diet (20%NS and 20%S) than in those fed 5% fat (5%NS and 5%S), and the feed efficiency ratio (body weight gain divided by feed intake during the experiment) was significantly higher in the 20% fat diet groups than in 5% fat diet groups. Cumulative energy intake of the rats was greatest in the 5%NS group, less in the 20%NS group, still less in the 5%S group, and least in the 20%S group; there was a signifi-
cant difference between the 5%NS group and the 20%S group. The results for the energy efficiency ratio (total body weight gain divided by energy intake during the experiment) were similar to those for body weight gain. Urinary excretion and PaA concentrations in the blood and organs of rats fed a PaA-restricted diet

Table 3 shows the amount of PaA excreted in the urine and the PaA concentrations in plasma, liver, muscle, heart, and adrenal glands. For urinary excretion of PaA in rats fed a PaA-restricted diet, there was a significant interaction between swimming and a high-fat diet by two-way ANOVA. Urinary PaA excretion was significantly lower in the 20%S group than in the 5% fat diet groups, and lower in the 20%S group than in all of the other groups. The plasma PaA concentration was significantly lower in the 20%S group than in the 5% fat diet groups. The concentrations of PaA in the heart were significantly higher in the swimming groups (5%S and 20%S) than in the 5%NS group. The concentrations of PaA in the adrenal glands were significantly higher in the swimming groups than in the non-swimming groups (5%NS and 20%NS). Liver and muscle concentrations of PaA did not differ among the groups.

The growth and PaA status of rats fed a PaA-sufficient diet

Table 4 shows the body weights on the initial, 15th, and final (37th) day of the experiment, and the body

| PaA-sufficient 5% fat diet | PaA-sufficient 20% fat diet |
|---------------------------|---------------------------|
| **Non-swimming**          | **Swimming**              | **Non-swimming** | **Swimming** |
| Initial body weight (g)   | 37.5±1.1                  | 37.9±1.2         | 37.5±0.8     | 37.5±1.1       |
| Body weight on day 15 (g) | 118±4                     | 120±2            | 120±5        | 119±3          |
| Final body weight (g)     | 273±4                     | 258±5            | 279±6*       | 260±7*         |
| Body weight gain (g/36 d) | 235±4                     | 221±4            | 241±5*       | 223±7*         |
| Feed intake (g/36 d)      | 510±12*                   | 491±10*          | 447±9b*      | 422±8b**       |
| Energy intake (MJ/36 d)   | 8.06±0.19                 | 7.88±0.12        | 8.46±0.09*   | 8.14±0.16*     |
| Feed efficiency ratio1   | 0.461±0.004a              | 0.453±0.004a     | 0.539±0.002b | 0.534±0.006b   |
| Energy efficiency ratio2  (g/MJ) | 29.0±0.5            | 28.9±0.2         | 27.9±0.1     | 27.9±0.3*      |

Values are means±SE (n=5).
1 Feed efficiency ratio, body weight gain (g/36 d)/feed intake (g/36 d).
2 Energy efficiency ratio, body weight gain (g/36 d)/energy intake (MJ/36 d).
Swimming×high-fat diet (interaction) was not significant for any value.
Values in the same row with different superscript letters are statistically different at p<0.05, as determined by Tukey’s multiple-comparison test.
Asterisks mark values which are statistically different at p<0.05, as determined by Student’s t-test, from those of the corresponding PaA-restricted group (Table 3).

| PaA-sufficient 5% fat diet | PaA-sufficient 20% fat diet |
|---------------------------|---------------------------|
| **Non-swimming**          | **Swimming**              | **Non-swimming** | **Swimming** |
| Urine (nmol/g of diet)     | 76.6±2.6ab                 | 68.6±2.6ab       | 60.2±4.2ab   | 56.1±8.5ab     |
| Plasma (nmol/mL)           | 3.44±0.07*                 | 3.31±0.12*       | 3.08±0.26*   | 2.81±0.11*     |
| Liver (nmol/g)             | 402±18                     | 386±13           | 429±12       | 388±19         |
| Muscle (nmol/g)            | 66.1±4.7*                  | 57.8±3.1*        | 49.0±4.8*    | 54.6±4.6*      |
| Heart (nmol/g)             | 198±4a*                    | 226±8bc*         | 207±2ab      | 233±7c*        |
| Adrenal glands (nmol/g)    | 83±5a                      | 133±10b          | 109±5ab      | 126±6b         |

Values are means±SE (n=5).
Swimming×high-fat diet (interaction) was not significant for any value.
Values in the same row with different superscript letters are statistically different at p<0.05, as determined by Tukey’s multiple-comparison test.
Asterisks mark values which are statistically different at p<0.05, as determined by Student’s t-test, from those of the corresponding PaA-restricted group (Table 3).
weight gain, total feed and energy intakes, and feed and energy efficiency ratios in rats fed a PaA-sufficient diet. The interaction between swimming and a high-fat diet was not significant for any measurement. In the rats fed a PaA-sufficient diet, body weight did not differ among the groups at any time point. Body weight gain, energy intake, and energy efficiency ratios also did not significantly differ. There were significant differences in feed intake and feed efficiency ratios between the 5% fat diet groups and the 20% fat diet groups. When each value was compared with PaA-restricted diet (e.g., initial body weight in PaA-restricted 5%NS vs in PaA-sufficient 5%NS), final body weight, body weight gain, feed and energy intake were significantly increased in PaA-sufficient 20% fat diet groups compared to the corresponding PaA-restricted groups. The energy efficiency ratio in PaA-sufficient 20%S increased compared with PaA-restricted 20%S.

Table 5 shows the urinary PaA excretion and the PaA concentrations in the plasma, liver, muscle, heart, and adrenal glands of rats fed sufficient PaA. The interaction between swimming and a high-fat diet was not significant for any value. Urinary PaA excretion was greatest in the 5%NS group, and progressively decreased in the 5%S group, 20%NS group, and 20%S group; urinary PaA excretion significantly differed between the 5%NS and 20%S groups. Plasma, liver, and muscle concentrations of PaA did not differ among the groups. The PaA concentrations in the heart were greatest in the 20%S group, less in the 5%S group, still less in the 20%NS group, and least in the 5%NS group. The concentration of PaA in the adrenal glands was significantly higher in the swimming groups than in the 5%NS group. Urinary excretion, plasma concentration, muscle and heart concentration in each PaA-sufficient group increased compared with the corresponding PaA-restricted group.

DISCUSSION

In this study, we evaluated the effects of exercise in combination with a normal (5%) or high (20%) fat diet on PaA status in rats fed a PaA-restricted or PaA-sufficient diet. The energy ratios for protein, fat and carbohydrate per total energy in the 5% fat vs. the 20% fat diet were 20% vs. 16%, 12% vs. 40%, and 68% vs. 44%, respectively. In Japan, these ratios are recommended to be within 13–20%, 20–30%, and 50–65%, respectively, for maintenance and promotion of human health (16). Thus, the energy ratios of the 5% fat diet are similar to those recommended for humans. Regarding the amount of PaA in diet, we have previously experimented with weaning rats which were fed a 5% fat diet with from 0 g to 0.016 g (amount contained in AIN-93 diet) PaA-Ca/kg diet for 28 d (13). The result indicated that 0.004 g PaA-Ca/kg diet gave maximum growth and maintained the PaA nutritional status. Therefore, in this study 0.004 g PaA-Ca/kg diet was defined as the required amount of PaA. To assess the requirement for PaA we used the urinary excretion of PaA, which reflects storage of vitamins in the body (17–19). If PaA intake is maintained at a constant level while PaA requirements increase, then urinary excretion of PaA decreases.

Excessive amounts of dietary fat are preserved as body fat in humans (20–22); similarly, a 20% fat diet is a risk factor for obesity in rats (23). However, in this study, the PaA-restricted rats in the 20%NS group had a lower final body weight than the rats in the PaA-restricted 5%NS (Table 2). This phenomenon was likely due to their restricted PaA intake and subsequent lack of PaA, which is necessary for the metabolism of excess dietary fat, and suggests that their PaA requirement was increased by their high-fat diet. Vitamin deficiencies of PaA have been shown to result in poor growth in rats (13, 24). In contrast, the rats fed the 20% fat diet containing a sufficient amount of PaA (PaA-sufficient 20%NS group) had a greater final body weight than the rats that received the restricted-PaA diet with 20% dietary fat, and did not have a lower final body weight than the rats fed the 5% fat diet (PaA-sufficient 5%NS) (Table 4). Although we did not test it here, we speculate that feeding rats a diet containing more than 0.016 g PaA-Ca/kg diet might result in greater body fat, and even obesity, because rats fed excess amounts of PaA would have more PaA available to metabolize and store excess dietary fat.

PaA is important for the synthesis and catabolism of fat. Although in this study urinary PaA excretion was not decreased by exercise except for influence of interaction, exercise increased fatty acid availability through catabolism of fat to provide energy. In contrast, PaA excretion was decreased by a high-fat diet, likely due to the increased preservation of fatty acids for the synthesis of body fat (Table 3). Achten and Jeukendrup (25) reported that fat oxidation during exercise was decreased by the ingestion of 75 g of glucose. The 5% fat diet was high in carbohydrate and low in fat, which may have resulted in decreased fat oxidation during exercise, and would not have increased utilization of PaA. Therefore, the swimming rats excreted as much PaA in their urine as the non-swimming rats. Fat synthesis might consume more PaA than fat catabolism, and the high-fat diet increased the requirement for PaA more than exercise did (Table 3). However, there have been no previous reports of this in the literature.

In the rats fed PaA-restricted diet, urinary excretion of PaA was synergistically decreased by the combination of a high-fat diet and exercise. Additionally, both plasma concentration of PaA and final body weight were decreased most in the rats in the group that was fed a high-fat diet combined with exercise compared with those exposed separately to a high-fat diet or exercise. Helge et al. (26) reported that fatty acid uptake and fat oxidation were increased by exercise combined with a high-fat diet. Thus, in our study, fat metabolism was not increased by exercise and little PaA was required by rats fed the 5% fat diet because there was little fat contained in the diet that could be metabolized for energy production. The 20% fat diet contained much more fat, which may have increased fat metabolism and PaA utilization in the exercised rats because the fat present in the diet was readily available. Therefore, the urinary excretion
and plasma concentration of PaA were synergistically decreased by the combination of a high-fat diet and exercise, and this led to the lower body weight gain and final body weight in Experiment 1. Furthermore, when the diet contained sufficient PaA, only urinary PaA was affected by the combination of high-fat diet and exercise (Tables 4 and 5). This confirmed that the urinary excretion of PaA decreased as the PaA requirement increased (as occurred in the 20% S group) in order to maintain growth and plasma PaA concentrations. These findings suggest that it is necessary to feed diets containing the minimum amount of vitamins when we want to reveal the effects of exercise on vitamin requirements.

The requirement for PaA was increased by the combination of a high-fat diet and exercise, and the urinary PaA excretion concomitantly decreased to preserve body PaA levels. Plasma (the vehicle for vitamin transport) concentrations of PaA also decreased, but PaA concentrations in the liver, which is a site of vitamin storage, were not decreased by a high-fat diet combined with swimming. Liver PaA concentrations in rats fed a PaA-restricted diet (Table 3) did not differ from the concentrations in rats fed a PaA-sufficient diet (Table 5). The adrenal glands, which synthesize steroid hormones from cholesterol, might preferentially take up PaA because of their role in fat metabolism. In our study, the PaA concentration in the adrenal glands did not differ between rats fed PaA-restricted and PaA-sufficient diets. The PaA concentrations in skeletal muscle and the heart were lower in rats fed PaA-restricted diets than in those fed PaA-sufficient diets, which suggests that these organs are less prone to preserve PaA than the liver and adrenal glands.

In this study, we found that the requirement for PaA in rats is synergistically increased with the combination of exercise and a high-fat diet. These results suggest that PaA intake should be increased in the presence of exercise or a high-fat diet, and especially when these conditions are combined. Intake of adequate PaA may prevent disorders characterized by a lack of PaA.

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Author contribution
KT and KS designed the research. KT conducted the research, and TF assisted in the research. KT and KS analyzed the data and drafted the manuscript. All authors approved the final manuscript. The authors declare that they have no conflict of interest.

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