

# The effects of exercise training and acute exercise duration on plasma folate and vitamin B<sub>12</sub>

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**BACKGROUND/OBJECTIVES:** Energy production and the rebuilding and repair of muscle tissue by physical activity require folate and vitamin B<sub>12</sub> as a cofactor. Thus, this study investigated the effects of regular moderate exercise training and durations of acute aerobic exercise on plasma folate and vitamin B<sub>12</sub> concentrations in moderate exercise trained rats.

**MATERIALS/METHODS:** Fifty rats underwent non-exercise training (NT, n = 25) and regular exercise training (ET, n = 25) for 5 weeks. The ET group performed moderate exercise on a treadmill for 30 min/day, 5 days/week. At the end of week 5, each group was subdivided into 4 groups: non-exercise and 3 exercise groups. The non-exercise group (E0) was sacrificed without exercising and the 3 exercise groups were sacrificed immediately after exercising on a treadmill for 0.5 h (E0.5), 1 h (E1), and 2 h (E2). Blood samples were collected and plasma folate and vitamin B<sub>12</sub> were analyzed.

**RESULTS:** After exercise training, plasma folate level was significantly lower and vitamin B<sub>12</sub> concentration was significantly higher in the ET group compared with the NT group ( $P < 0.05$ ). No significant associations were observed between plasma folate and vitamin B<sub>12</sub> concentrations. In both the NT and ET groups, plasma folate and vitamin B<sub>12</sub> were not significantly changed by increasing duration of aerobic exercise. Plasma folate concentration of E0.5 was significantly lower in the ET group compared with that in the NT group. Significantly higher vitamin B<sub>12</sub> concentrations were observed in the E0 and E0.5 groups of the ET group compared to those of the NT group.

**CONCLUSION:** Regular moderate exercise training decreased plasma folate and increased plasma vitamin B<sub>12</sub> levels. However, no significant changes in plasma folate and vitamin B<sub>12</sub> concentrations were observed by increasing duration of acute aerobic exercise.

Nutrition Research and Practice 2016;10(2):161-166; doi:10.4162/nrp.2016.10.2.161; pISSN 1976-1457 eISSN 2005-6168

**Keywords:** Folic acid, vitamin B<sub>12</sub>, physical activity, aerobic exercise, rodent

## INTRODUCTION

The B-vitamins are particularly important for physical exercise because they are involved in regulation of energy metabolism by modulating the synthesis and degradation of carbohydrates, fats, and proteins. Folate, one of the B-vitamins, is reduced to tetrahydrofolate, serving as an essential cofactor in methylation reactions, including the vitamin B<sub>12</sub>-dependent formation of methionine from homocysteine, and as a carrier of one-carbon units involved in the synthesis of purines and pyrimidines. The roles of folate and vitamin B<sub>12</sub> in assisting with cell division make them critical nutrients for growth, synthesis of new cells, such as red blood cells, and for the repair of damaged cells and tissues [1]. Many methylated molecules (e.g., acetylcholine, creatine, and DNA) are essential for physical activity [2-4]. The exercise-induced metabolic demand possibly exerts an increased turnover of many of these substrates. It can be supposed that regeneration of these substrates is accompanied by considerable stimulation of the methionine metabolism [5]. In the degradation

of amino acids such as valine, isoleucine, methionine, and threonine and fatty acids, these amino acids are converted to propionyl-CoA and fatty acids are oxidized to acetyl-CoA and propionyl-CoA. Acetyl-CoA enters directly into the tricarboxylic acid (TCA) cycle. Propionyl-CoA is carboxylated to methylmalonyl-CoA and finally converted to succinyl-CoA by methylmalonyl-CoA mutase. This enzyme requires vitamin B<sub>12</sub> as a cofactor [6]. Succinyl-CoA is used for energy production in the TCA cycle. Thus, folate and vitamin B<sub>12</sub> status and requirements may be altered by energy production and rebuilding and repair of muscle tissue induced by physical activity.

Acute exercise may cause muscular damage and accelerate protein catabolism, which will lead to an increase of muscular amino acid pools and homocysteine production in the methionine metabolism [7].  $\beta$ -oxidation of fatty acids is a minor component of energy expenditure early in exercise, however sustained exercise at low or moderate intensity requires a substantial contribution of energy from fat oxidation. Physical exercise demands a significant amount of energy turnover and

This research was supported by the 2014 research fund from Duksung Women's University.

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Received: December 1, 2015, Revised: January 11, 2016, Accepted: February 14, 2016

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the metabolic pathways for energy production are stressed during exercise. Exercise training induces adaptations to stress during exercise and plays a key role in homeostasis related to an increase in aerobic metabolism [8-9]. Endurance exercise training resulted in increased plasma free fatty acids and free fatty acid oxidation and reduced glucose oxidation [10]. The effects of exercise training in increases of body turnover or requirement of several micronutrients including thiamin, riboflavin, and vitamin B<sub>6</sub> have been reported [11-14]. However, little information is available on the effects of exercise training and acute physical activity on folate and vitamin B<sub>12</sub> status.

The aim of this study was to examine the influence of 5-week regular moderate exercise training and duration of acute aerobic exercise on plasma folate and vitamin B<sub>12</sub> concentrations in rats. We hypothesized that acute aerobic exercise can decrease plasma folate and vitamin B<sub>12</sub> concentrations since folate and vitamin B<sub>12</sub> are used as co-factors of methionine and lipid metabolism and the magnitude of this decrease might differ according to regular moderate exercise training and duration of acute exercise.

## MATERIALS AND METHODS

### Experimental animals and diets

Fifty 4-week old male Sprague-Dawley rats (Daehanbiolink Co., South Korea) weighing 95-105 g were fed a diet which met the AIN-93 recommendations (American Institute of Nutrition). The AIN-93 diet contained 2 mg/kg diet of folate and 25 mg/kg of vitamin B<sub>12</sub>, respectively. The folate and vitamin B<sub>12</sub> intakes of rats were calculated with the amount of feeding and the composition of the diet. Animals were kept individually in stainless-steel cages in a room controlled for temperature (22 ± 1°C), relative humidity (50-60%), and light (12-hour light/dark cycle). The study protocol was approved by the Committee on Animal Welfare Regulations of Duksung Women's University, South Korea (2011-108).

### Exercise and collection of samples

Fifty animals were randomly assigned to one of two groups: non-exercise training (NT, n = 25) or regular exercise training (ET, n = 25) for 5 weeks. For exercise training, the rats performed moderate exercise on a treadmill (Jungdo Bio & Plant, JD-A-09,

South Korea, 15° incline, 0.5-0.8 km/h) for 30 minutes per day and 5 days per week. Rats who did not engage themselves in running on the treadmill were forced to exercise using light electric shocks. [16]. At the end of week 5, the rats were subdivided into four groups based on exercise duration: non-exercise (0 h (E0), n = 4) and 3 exercise groups (0.5 h (E0.5), 1 h (E1), and 2 h (E2), n = 7 per group). The E0 groups were sacrificed without having performed exercise at the end of week 5. The 3 exercise groups were sacrificed immediately after exercising on a treadmill (15° incline, 0.5-0.8 km/h) for 0.5 h, 1 h, and 2 h, respectively. No deaths occurred during or after exercise in the exercise groups. At each of the respective time points the animals were sacrificed by decapitation under light ether anesthesia. Blood samples were collected and centrifuged at 3,000 rpm for 10 minutes at 4°C. All plasma samples were stored at -70°C until analysis (Fig. 1).

### Biochemical analysis

Plasma folate levels were determined by a microbiological method using *Lactobacillus casei* (ATCC 7469) with a 96-well microplate reader and each sample was analyzed in triplicate [15]. Vitamin B<sub>12</sub> concentrations in plasma were analyzed by an enzyme immunoassay using a commercially available kit (Biotain Pharma Co., Ltd., China). Plasma folate levels were expressed as nmol/L and vitamin B<sub>12</sub> levels as pmol/L.

### Statistical analysis

Data were subjected to an analysis of variance (ANOVA) and followed by Duncan's multiple range test using SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina, US) to determine differences by durations of acute aerobic exercise. In addition, differences between the ET and NT groups were determined using t-test. Pearson's correlation coefficients were calculated to determine correlations between intake and plasma concentrations of folate and vitamin B<sub>12</sub> and to find a relationship between plasma folate and vitamin B<sub>12</sub> concentrations. Results were considered statistically significant at  $P < 0.05$ .

## RESULTS

### Body weight and folate and vitamin B<sub>12</sub> intakes

No difference in the initial body weight was observed

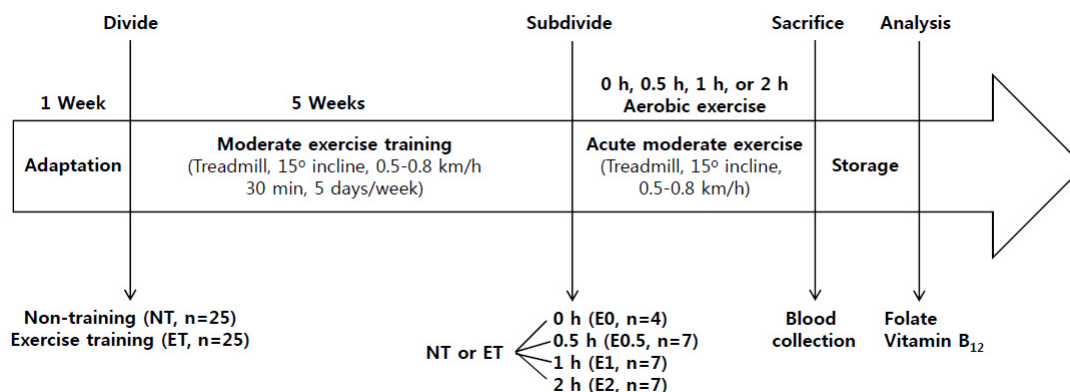


Fig. 1. Schematic overview of experimental design

**Table 1.** Effect of regular exercise training on body weight, feed intake, folate intake, and vitamin B<sub>12</sub> intake

	Group		P-value
	NT <sup>1)</sup> (n = 25)	ET <sup>2)</sup> (n = 25)	
Initial body weight (g)	96.00 ± 3.25	95.22 ± 3.91	0.4463
Final body weight (g)	452.00 ± 31.56	417.20 ± 32.93	0.0004***
Average weight gain (g/day)	8.48 ± 0.76	7.67 ± 0.79	0.0006***
Feed intake (g/day)	23.36 ± 1.65	22.50 ± 1.86	0.0895
Folate intake (µg/day)	46.72 ± 3.31	45.00 ± 3.72	0.0895
Vitamin B <sub>12</sub> intake (µg/day)	584.05 ± 41.36	562.47 ± 46.54	0.0895

Values are expressed as mean ± SD, \*\*\* significant at  $P < 0.001$  by t-test

<sup>1)</sup> Non-training group

<sup>2)</sup> Exercise training group

**Table 2.** Effect of regular exercise training on plasma folate and vitamin B<sub>12</sub> concentrations

	Group		P-value
	NT <sup>1)</sup> (n = 25)	ET <sup>2)</sup> (n = 25)	
Plasma folate (nmol/L)	208.5 ± 23.80	192.3 ± 31.70	0.0467*
Plasma vitamin B <sub>12</sub> (pmol/L)	613.6 ± 56.47	659.0 ± 42.33	0.0023**

Values are expressed as mean ± SD, \*  $P < 0.05$  \*\*  $P < 0.01$  by t-test

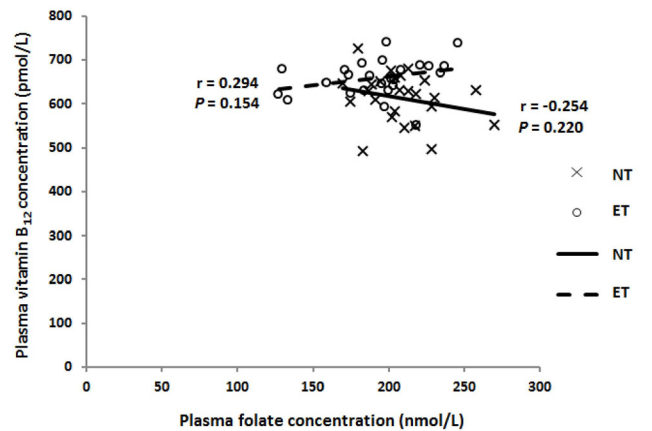
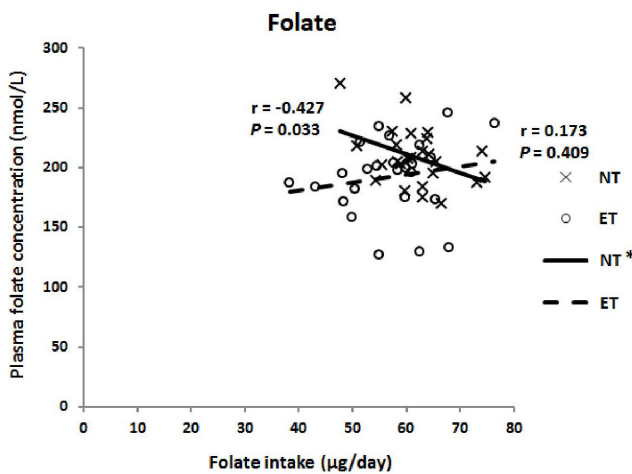
<sup>1)</sup> Non-training group

<sup>2)</sup> Exercise training group

between the NT and ET groups; however, the final body weight and average weight gain were significantly lower in the ET group compared with those of the NT group (Table 1). No significant differences in folate and vitamin B<sub>12</sub> intake were observed between the groups.

*Plasma folate and vitamin B<sub>12</sub> concentrations*

After regular exercise training for 5 weeks, plasma folate level was significantly lower in the ET group compared with the NT group ( $P < 0.05$ ), however plasma vitamin B<sub>12</sub> concentration was significantly higher in the ET group than in the NT group ( $P < 0.01$ ) (Table 2).



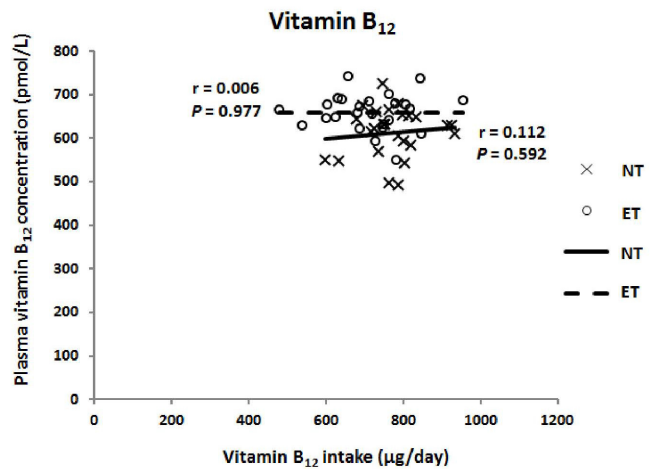
**Fig. 3.** Effect of regular exercise training on the associations between plasma folate and plasma vitamin B<sub>12</sub> concentrations. No significant correlations were observed by Pearson's correlation coefficient. NT: non-training group (n = 25) and ET: exercise training group (n = 25)

*Associations between intake and plasma concentration of folate and vitamin B<sub>12</sub> and between plasma folate and vitamin B<sub>12</sub> concentrations by regular exercise training*

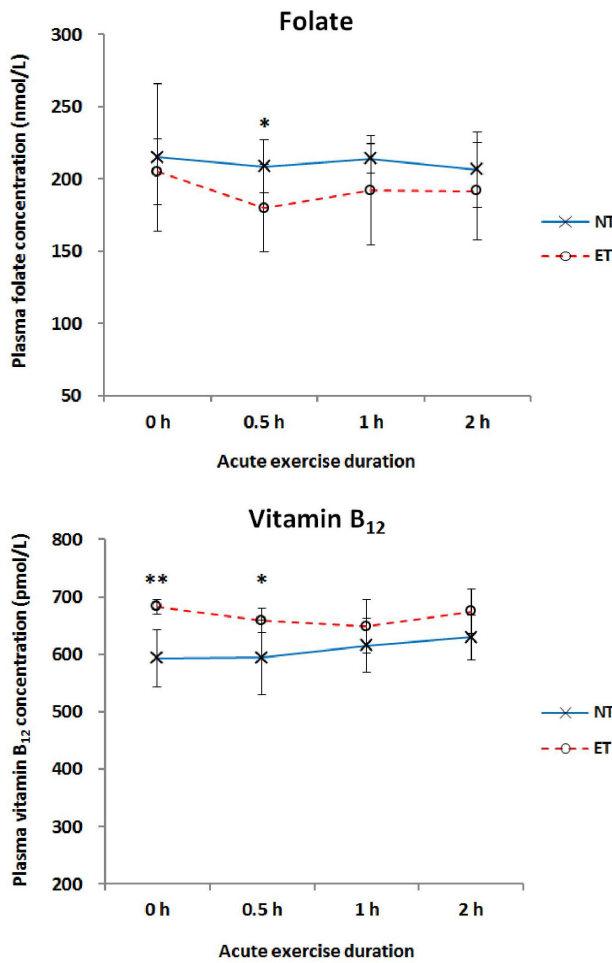
Plasma folate concentrations were significantly decreased by dietary folate intake only in the NT group ( $r = -0.427$ ,  $P = 0.033$ ). No significant associations between dietary intake and plasma concentration of vitamin B<sub>12</sub> were observed in both the NT and ET groups (Fig. 2). No associations were observed between plasma folate and vitamin B<sub>12</sub> concentrations in the NT group ( $r = -0.254$ ,  $P = 0.220$ ) and the ET group ( $r = 0.294$ ,  $P = 0.154$ ) (Fig. 3).

*Plasma folate and vitamin B<sub>12</sub> concentrations by durations of acute aerobic exercise*

No differences of plasma folate levels by durations of aerobic exercise were observed in the NT group (Fig. 4). In the ET group, plasma folate level was the lowest in the E0.5 group; however, there was no significant difference by exercise duration. Plasma folate was significantly lower in the E0.5 group in the ET group than in the NT group ( $P < 0.05$ ).



**Fig. 2.** Effect of exercise training on the associations between intake and plasma concentration of folate and vitamin B<sub>12</sub>. NT: non-training group (n = 25) and ET: exercise training group (n = 25)



**Fig. 4.** Effect of acute exercise on plasma folate and vitamin B<sub>12</sub> concentrations by exercise training. \*  $P < 0,05$ , \*\*  $P < 0,01$  by t-test at each duration (0 h, n = 4; 0,5 h, n = 7; 1 h, n = 7; and 2 h, n = 7) between the non-trained (NT) and exercise trained (ET) groups.

In both the NT and ET groups, plasma vitamin B<sub>12</sub> concentrations were not significantly changed by increasing exercise duration. Significantly higher vitamin B<sub>12</sub> concentrations were observed in the E0 and E0.5 groups of the ET group compared to those of the NT group, respectively. No differences in plasma vitamin B<sub>12</sub> levels at the E1 and E2 were observed between the ET and NT groups.

**DISCUSSION**

This study showed that regular aerobic exercise training for 5 weeks altered plasma folate and vitamin B<sub>12</sub> concentrations. However, plasma folate and vitamin B<sub>12</sub> concentrations were not altered under different durations of acute aerobic exercise in both NT and ET groups.

Physical activity influences protein metabolism and turnover and thus the concentration of certain amino acids, including methionine [16]. Methionine concentration in plasma increased significantly during exercise and methionine concentration was shown to decrease below basal levels hours after exercise in

both humans and rats [18-19]. This finding suggests that decreased plasma methionine after exercise may be due to increased muscle anabolism requiring methionine thus leading to reduced substrate for transmethylation reactions and homocysteine concentration [18,20]. Thus, individuals who participate in regular exercise training may have less folate and vitamin B<sub>12</sub> available for methionine metabolism. In this study, we hypothesized that regular moderate aerobic exercise training significantly reduced plasma folate concentration in rats, which may be due to an increased use of folate for methylation including methionine metabolism by physical activity. However, vitamin B<sub>12</sub> level was significantly higher in the ET group than in the NT group. In this study, folate and vitamin B<sub>12</sub> intakes were similar in the NT and ET groups. Thus, other adaptation mechanisms for exercise training may affect plasma vitamin B<sub>12</sub> concentration.

Endurance exercise training results in sparing of plasma glucose and liver glycogen by increasing the oxidation of fat during submaximal exercise [21]. After training, the decreased carbohydrate utilization during exercise is compensated for by an increase in fat oxidation [22]. Mild- or moderate-intensity exercises are associated with a 5-10-fold increase in fat oxidation above resting amounts [23]. The increased capacity to use fat following exercise training results from an enhanced ability to mobilize free-fatty acids from fat depots and an improved capacity to oxidize fat consequent to the increase in the muscle enzymes responsible for fat oxidation [24]. Choi and Cho [25] reported that aerobic exercise trained rats had significantly higher plasma free-fatty acid compared to sedentary rats. The function of vitamin B<sub>12</sub> is different from that of folate, which is involved in fat oxidation by acting as a cofactor of methylmalonyl-CoA mutase. Therefore, in this study, an increase in plasma vitamin B<sub>12</sub> concentration in the ET group may be due to increased fat oxidation as an exercise adaptation.

In this study, plasma folate and vitamin B<sub>12</sub> concentration were also determined by different durations of acute aerobic exercise in the NT and ET groups. Plasma folate concentrations in both groups were not changed by acute exercise duration although there were significant alterations in folate concentration after 5-week training. The acute effect of exercise on protein turnover is catabolic, thus total plasma concentration of amino acids and sulfur-containing amino acids as well as homocysteine is increased after acute physical activity [26]. Thus, during acute exercise, the metabolism requiring folate may not be promoted by increasing exercise duration. However, long-term effects are an overall increase in protein synthesis and lean body mass [27]; therefore, after exercise training, the folate requirement may be increased for formation of methionine from increased homocysteine by acute exercise for protein synthesis and lean body mass. Although vitamin B<sub>12</sub> level of each group was not altered significantly by acute exercise duration, vitamin B<sub>12</sub> level of the NT group tended to increase by duration. Before exercise and after 0.5-hour acute aerobic exercise, vitamin B<sub>12</sub> concentrations were significantly lower in the NT group compared with those of the ET group; however there were no differences in vitamin B<sub>12</sub> concentrations after 1-hour exercise. The study conducted by Choi and Cho [25] reported that after 1-hour acute aerobic exercise, plasma free-fatty acids in both exercise

trained and sedentary rats were similar although the concentration in exercise trained rats was higher than that in sedentary rats before exercise. Thus, in the current study, an increase in plasma free-fatty acid by aerobic exercise may demand a vitamin B<sub>12</sub> influx into plasma from the stored vitamin B<sub>12</sub> in the liver for fat oxidation in the NT group.

However, the results of human studies regarding the effects of exercise on plasma folate and vitamin B<sub>12</sub> levels are equivocal. Plasma folate was increased during a 3-week training (swimming) period, but dropped after 5 days of recovery training [7]. In physically active subjects, erythrocyte folate and serum vitamin B<sub>12</sub> were stable in both a normoxia- and hypoxia-training for 4 weeks [28]. In soccer players, plasma folate concentrations before and after acute high-intensity sprint exercise were similar, but vitamin B<sub>12</sub> concentration after exercise was significantly higher than that before exercise [29]. Individuals involved in high intensity physical activity for at least 5 years had slightly higher plasma vitamin B<sub>12</sub> concentrations than sedentary individuals [30]. The plasma B<sub>12</sub> levels at 6 months in exercising (brisk walking for 20-60 min) women did not differ from those in a control group of similarly afflicted women who did not exercise [31]. Also, vitamin B<sub>12</sub> concentrations were not significantly influenced either by 4 weeks of strenuous endurance training or by differences in duration of acute exercise in trained male triathletes [32]. Most human studies were conducted with an uncontrolled diet during experiments and the subjects were already trained before exercise training or acute exercise for the studies, which might have induced the different results. No rat study to find the effects of exercise training and acute exercise on plasma folate and vitamin B<sub>12</sub> has been reported.

In conclusion, regular moderate exercise training decreased plasma folate level, but increased vitamin B<sub>12</sub> concentration, which might result from the adaptation for exercise training. By increasing duration of acute aerobic exercise, no significant alterations of plasma folate and vitamin B<sub>12</sub> concentrations were observed in the NT and ET groups. Further research is needed in order to examine change of folate and vitamin B<sub>12</sub> requirements and necessity for supplementation of the vitamins during exercise training and to determine the association of vitamin B<sub>12</sub> and fat oxidation for body adaptations of aerobic exercise training.

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