Elevation of Blood NAD Level after Moderate Exercise in Young Women and Mice

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Summary We previously reported that the blood NAD levels are decreased by severe exercise, and administration of nicotinamide, a precursor of NAD, improves the endurance capacity of mice. In the present study, we determined whether moderate exercise changes the blood NAD levels in humans and mice. College female students exercised moderately with bike-ergometers. The blood NAD levels elevated after moderate exercise. Mice were forced to swim in a running water pool for 5 min as a moderate exercise, 15 min as a strong exercise, and until exhaustion as a severe exercise (average swimming time was 28.7 min). A 5 min swim gave a result similar to that of moderate exercise by human subjects. However, the blood NAD levels decreased after all-out exercise. The changes in whole blood tryptophan (a precursor of pyridine nucleotides) levels were similar to that in NAD. The glucose levels in whole blood and the non-esterified fatty acid levels in serum decreased according to exercising time. These data are the first demonstration of moderate exercise raising the blood NAD levels in human and mice. Elevation of the blood NAD levels may reflect changes in niacin metabolism that occur in response to exercise.

Key Words NAD, NADP, tryptophan, exercise, blood

Recently, humans have been taking exercise such as jogging, swimming, bike-ergometer exercise and motor-driven treadmill running to maintain health and also to improve metabolic disorders. Exercise requires extra-energy expenditure, but it also increases a loss of micronutrients such as vitamins and minerals. In particular, exercise increases the requirements for thiamin, riboflavin, and niacin; therefore, the Recommended Dietary Allowances of these vitamins are laid down on the basis of energy intake (1). Some investigators have reported that supplements of thiamin (2), riboflavin (3), and niacin (4) improve exercise performance. As for niacin, NAD (NAD+ and NADH) is important in the formation of ATP, since three molecules of ATP are reformed by the mitochondrial electron transport system and oxidative phosphorylation from one molecule of NADH. However, little is known about the behavior of NAD levels in blood during exercise. The blood NAD levels, a sensitive indicator for the assessment of niacin status (5–7), decreased when rats were exhausted by swimming (4). We report here the change of blood NAD level during exercise in humans and mice.

In a human bike-ergometer exercise experiment, the subjects (n=43) were college female students, who accepted our experimental object and plan. Each subject signed an informed consent document prior to her participation, and the experiments were approved by the Committee of The University of Shiga Prefecture for the Ethical Treatment of Human Subjects. The subjects took self-selected food at 12:00–12:30 and exercised on bike-ergometer at 15:00–16:00. The exercise was programmed as shown in Fig. 1. The heart rate was ca. 180/min at the end of exercise and the total energy expenditure was ca. 100 kcal. A 10-μL sample of finger blood was taken prior to exercise and right after exercise. The NAD (NAD++NADH) and NADP (NADP++NADPH) were measured by the method of Shibata and Murata (8), and Shibata and Tanaka (9), respectively. The elevation of blood NAD level was observed by the moderate exercise as shown in Table 1. Even at recovery, it was still higher than the value of pre-exercise. The blood NADP level also increased by the moderate exercise (Table 1). At recovery, it returned to the pre-exercise level.

In an animal experiment, male mice of Slc:ICR strain (10-wk-old; 38–40 g of body weight) were purchased from SLC (Hamamatsu, Japan). The mice were housed in standard wire mesh cages (33×23×12 cm; 5 mice/cage) in a vivarium maintained at 20±2°C under a 12:12 h light : dark cycle (light onset at 06:00 h) and around 50% humidity. A commercial standard diet (MF; Oriental Yeast Co., Tokyo, Japan) and water were available ad libitum. The care and treatment of the experimental animals conformed to The University of Shiga Prefecture Guideline for the Ethical Treatment of Laboratory Animals. The mice were maintained for the...
first 8 days after arrival (the arrival day was designated 0) to acclimate them to their new surroundings. The mice were forced to swim twice for 5 min at the flow-rate of 6 L/min as a training at 14:00–15:00 on days 9 and 12. The exercise was carried out using an adjustable-current swimming pool described previously (10). They were then divided into four groups at random (non-, 5 min-, 15 min-, and all-out swimming groups) at 14:00 on day 15, and forced to swim at the flow-rate of 8 L/min. The mice were killed right after exercise by decapitation and samples of whole blood from the carotid arteries were collected into microtubes. The samples of collected whole blood were immediately used for measuring NAD (7), NADP (8), Trp (11), serotonin (12), and glucose, which was measured by the method of “Glucose-TestWako” (code No. 273-13901) obtained from Wako Pure Chemicals Industries (Osaka, Japan). In order to get serum for measuring non-esterified fatty acids (NEFA), which was measured by the method of “NEFA-HA TestWako” (code No. 276-80502) obtained from Wako, the samples of whole blood were left at room temperature for 2 h and the blood clots formed in the samples were then removed. The resulting liquids were left for 30 min at 37°C and then for 60 min at 4°C, and they were centrifuged for 10 min at 1,000×g at 4°C. The resulting supernatants were used for measuring NEFA.

The blood NAD levels were elevated by moderate exercise (5-min swimming), returned to the non-exercise levels after a strong exercise (15-min swimming), and decreased after severe exercise (28.7 ± 9.5 min swimming) as shown in Table 2. The decrease in NAD levels was also observed in rats when they were forced to swim until exhaustion (4). The blood NADP levels tended to rise after moderate and strong exercises, and returned to the non-exercise levels after all-out exercise (Table 2). The blood NAD levels were 4.5-times higher in mice than in humans but the blood NADP levels were almost the same. The Trp, a precursor of NAD and NADP, in whole blood was transiently elevated by moderate exercise, and decreased after strong exercise, similar to that in NAD (Table 2). The serotonin, a Trp metabolite, in whole blood did not change by exercise (Table 2). The blood glucose and NEFA levels decreased according to the degree of exercise (Table 2).

The blood NAD content can be a relevant biomarker of niacin status (5–7), and is affected by niacin nutrition (6, 7) and malaria (13). Therefore, the effects of ex-
exercise on niacin metabolism can be known by measuring the blood NAD content. The liver and kidneys can synthesize NAD from nicotinamide, nicotinic acid and Trp, whereas other tissues including skeletal muscle and erythrocyte synthesize NAD only from nicotinamide supplied from the liver (14, 15). Thus, the liver and/or kidneys play a major role in niacin metabolism. In the present study, the severe exercise decreased the blood NAD content in mice (Table 2). We previously reported that the administration of nicotinamide, a precursor of NAD, elongated the marginal swimming period, and prevented the decrease of blood NAD levels (4). These findings suggest that a large amount of NAD is spent to form ATP during exercise in skeletal muscle, and NAD plays an important role to supply energy during exercise. Interestingly, this study showed that the moderate exercise increased the blood NAD content in both humans and mice (Tables 1 and 2). This result suggests that exercise changes the niacin metabolism. Ito et al. reported that exercise increased serum kynurenine, an intermediate of the biosynthetic pathway of niacin from Trp, suggesting the influence of exercise on the Trp metabolism (16). Although the mechanism by which exercise increases the blood NAD content is not clear, exercise may induce the biosynthesis of niacin from Trp, suggesting the influence of exercise on the de novo biosynthesis of niacin (17). Determining whether the blood Trp level affects the conversion ratio of Trp to niacin will be an important focus for future investigation.

In summary, these studies provide the first evidence that moderate exercise increases the blood NAD levels in human and mice. Furthermore, these are the first studies to show that the blood NAD levels can be increased by a normal physiological stimulus. Because the blood NAD content can be a relevant biomarker of niacin status, the elevation of blood NAD levels may reflect changes in niacin metabolism that occur in response to exercise.

REFERENCES