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Changes in Serum Hypoxanthine Levels after Walk Loads at Mild to High Intensity in Healthy Humans

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Saiki, S., Sato, T., Agatuma, H., Igarashi, T., Hiwatari, M. and Harada, T. *Changes in Serum Hypoxanthine Levels after Walk Loads at Mild to High Intensity in Healthy Humans.* Tohoku J. Exp. Med., 1999, **188**(1), 61-69 —— Effect of mild intensity exercise on the serum levels of hypoxanthine was studied. Eighteen healthy subjects performed 2 to 4 bouts of 5 minutes walk load at different intensities. At the beginning, thirteen of them walked at intensity more than 80% of the maximum. The serum levels of hypoxanthine increased to the levels of more than 6 times of resting values showing a peak at 10 to 20 minutes after the completion of the walk load. In 62 bouts of the walk load by 18 subjects, statistically significant relationship was demonstrated between intensity of the walk load and increase in serum concentration of hypoxanthine at 10 minutes after the completion of the walk load with correlation coefficient of 0.556. The serum hypoxanthine levels were significantly increased by the walk load even at mild intensity between 41 and 60%. Increment in the serum hypoxanthine concentration also showed positive and statistically significant correlation with physiological cost index. These results suggest that the serum levels of hypoxanthine increase following mild as well as moderate to submaximal intensity of exercise, and its increment may be used as an indicator of energy balance in the muscle during exercise at mild to high intensity. ———— hypoxanthine; exercise; physical activity; physiological cost index

Consumption of muscle adenosine triphosphate (ATP) is pronounced by intense exercise or ischemia, which is followed by formation of hypoxanthine via adenosine monophosphate (AMP) and inosine monophosphate (IMP). Hypoxanthine diffuses slowly from muscle to blood stream, and a part of it is converted to uric acid forming free radicals in the oxidative process. Therefore, the serum level of hypoxanthine is thought to be an extracellular metabolite monitoring the

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intracellular energy metabolism (Murray 1971), an indicator of tissue hypoxia (Saugstad et al. 1976, 1977; Sorlie et al. 1982), and a marker of free radical formation after reperfusion (Sahlin et al. 1991). Many studies demonstrated that hypoxanthine is produced in muscles during moderate to intense exercise, and there is great individual variation of changes in plasma concentrations of hypoxanthine by intense exercise (Ketai et al. 1987; Sahlin et al. 1991; Bangsbo et al. 1992; Hellsten et al. 1993b). Ketai et al. (1987) reported that the plasma levels of hypoxanthine are unchanged after low intensity exercise, even lasting for a prolonged time period, and increased after exercise at moderate to submaximal intensity.

Several studies demonstrated that muscle contents of metabolites are changed by mild intensity exercise. Sahlin and Katz (1989) reported that exercise at intensity at 40% of VO_2 maximum decreased muscle contents of creatine phosphate without increase of inosine monophosphate IMP. According to Green et al. (1983), muscle lactate was increased as well as blood lactate and muscle ATP showed tendency to be decreased by mild exercise at intensity below anaerobic threshold. Furthermore, McCully et al. (1988) demonstrated by ^{31}P magnetic resonance spectroscopy spectra that muscle creatine phosphate and ATP were decreased by mild intensity of exercise. These results suggest that the serum hypoxanthine levels may be increased by even mild exercise.

The purpose of the present study was to investigate effect of especially mild intensity of exercise on the serum levels of hypoxanthine.

Methods

Subjects and exercise test

After informed consent, 18 healthy male subjects with mean age of 27 ± 12 years were enrolled in the study. After resting for at least 20 minutes, they walked for 5 minutes around a square of 30 m length. They performed the walk load 2 to 4 times at different intensities from mild to submaximal on the different days. Heart rate was monitored by heart rate monitor (DS-1033B, Fukuda Co., Tokyo) before and during the walk load. Intensity of exercise was estimated by heart rate response, calculating ratio of increase of heart rate (the difference of heart rate between at rest and during exercise) to the predicted maximal heart rate, which was estimated by drawing number of age from 220. Exercise intensity was fixed at the same level during each bout. Physiological cost index (PCI) was calculated dividing the increment of heart rate during the walk load by walking speed (Steven et al. 1983).

Analysis

Venous blood samples were drawn from the anticubital vein prior to and at 10, 20, and 30 minutes after the completion of the walk load.

Blood was centrifuged immediately in serum separation tube Bunrimate MF

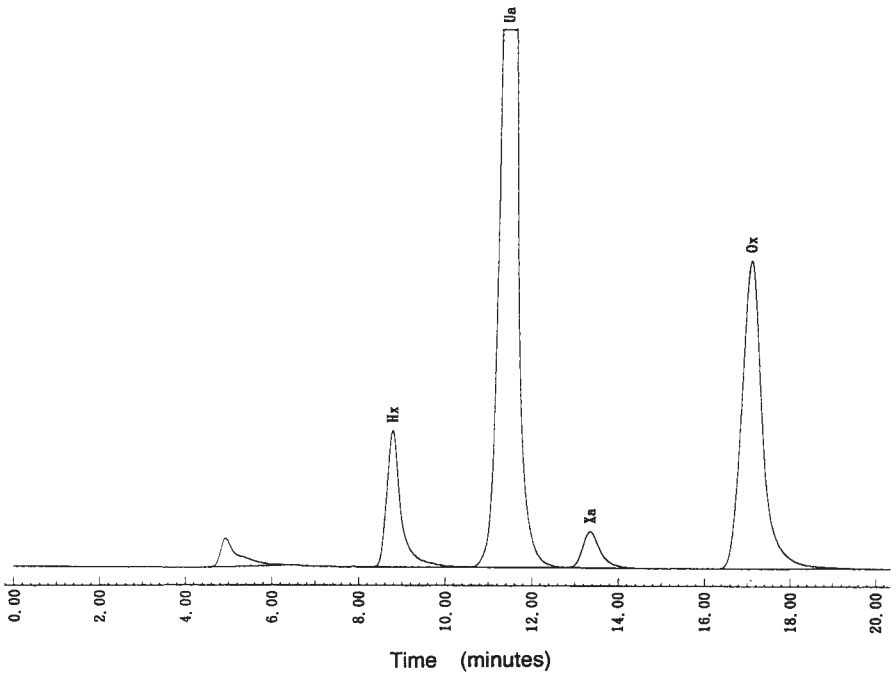

Fig. 1. Chromatogram of the mixed standard of hypoxanthine (Hx), uric acid (Ua), xanthine (Xa) and an internal standard oxypurinol (Ox), monitored at 280 nm.

(ONO Pharmaceutical Co., LTD, Osaka), serum was removed, and stored at $-20^{\circ}C$ until assayed. Oxypurinol was added to each sample as an internal standard before analysis. Twenty micromoliter of standard solution or serum was applied to high-performance liquid chromatography and analyzed by a modified method of Wung and Howell (1980) described by Hellsten-Westing et al. (1989) with Gilson-305 equipped with Gilson UV Master-1001 and system work station Model MS-712 (Gilson Medical Electronics, Inc., Middleton, WI, USA). Column of SynChropak RP-P-100 (250×2.1 mm I.D., 100Å, Micra Scientific Inc., Northbrook, IL, USA) was used as a separation column, and SynChropak RP-P-300 (50×4.6 mm I.D., 300Å, Micra Scientific Inc.) as a guard column. Hypoxanthine was separated under isocratic condition with the phosphate buffer as eluent, at a flow rate of 0.25 ml/minute (Fig. 1). Standard curve was made for 3.7 to 18.5 μM of hypoxanthine, and correlation coefficient between area ratio to oxypurinol and concentration of hypoxanthine was 0.999.

Statistics

Statistical analyses were performed with a commercially available program (Cricket Graph III Version 1.01; Computer Associates International, Inc., Islandia, NY, USA) by Macintosh Classic personal computer (Apple Computer, Cupertino, CA, USA), and $p < 0.05$ was considered significant. Values are given in terms of mean $\pm$ S.D.

Results

The time/concentration curves for serum hypoxanthine after the bouts of the walk load at intensity more than 80% are shown in Fig. 2. Changes in the serum levels of hypoxanthine following the bouts showed a great individual variation among subjects, showing peak at 10 to 20 minutes after the completion of the walk load in the most subjects. The mean of the peak hypoxanthine level and values

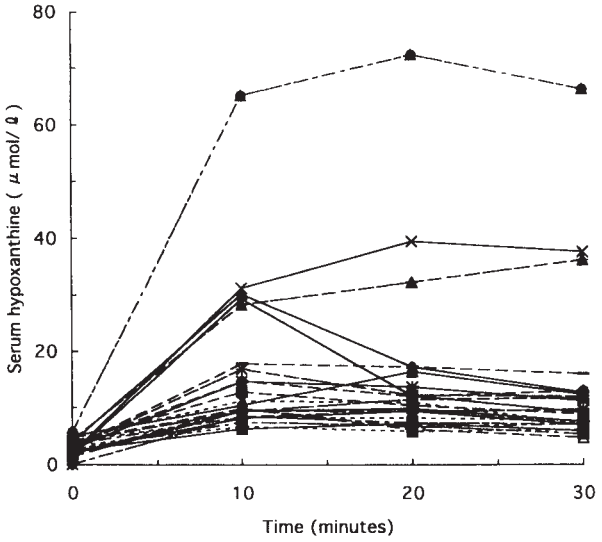

Fig. 2. Changes in serum concentration of hypoxanthine after walk in 18 healthy subjects. They walked at the maximal speed for 5 minutes. Intensity of exercise estimated by change of heart rate ranged from 77.9 to 95.0% of the maximum. Elevation of the serum levels of hypoxanthine after exercise showed a great individual variation.

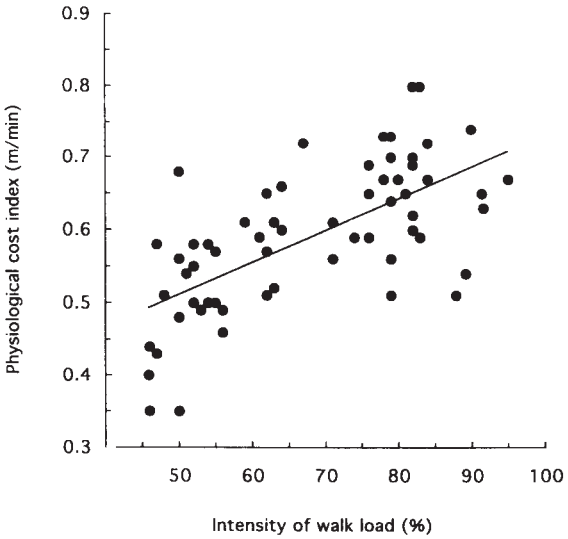

Fig. 3. Intensity of exercise and physiological cost index. Eighteen healthy subjects walked for 5 minutes at various intensity. Intensity of exercise varied from 29.0 to 95.0% of the maximum, which was estimated by changes in heart rate. Correlation equation between them was $Y=0.291+0.0044X$ ($r=0.653$, $p<0.01$)

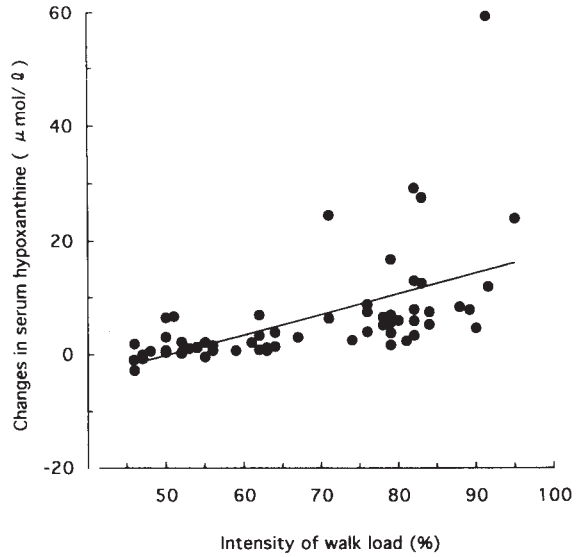

Fig. 4. Relations between changes in serum hypoxanthine and exercise intensity. The serum hypoxanthine levels 10 minutes after the completion of the walk at various intensities for 5 minutes are shown for 18 subjects. Correlation equation between them was $Y = -18.148 + 0.3609X$ ($r = 0.556$, $p < 0.001$).

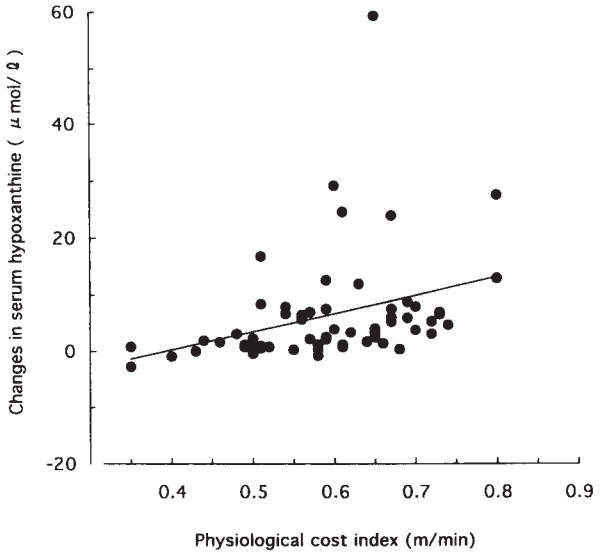

Fig. 5. Relations between changes in serum hypoxanthine and physiological cost index. Correlation equation between them was $Y = -12.746 + 32.457X$ ($r = 0.338$, $p < 0.01$).

at 10 minutes after the completion of the walk load were 20.2 ± 18.1 and 18.8 ± 15.2 μmol/liter, respectively, which were significantly greater than the resting level of 2.9 ± 1.5 μmol/liter ($p < 0.01$).

In 62 bouts by 18 subjects, they walked 466 to 1008 m at intensity of 45.9 to 95.0%. Intensity of exercise and PCI showed significant positive correlation with correlation coefficient of 0.653 (Fig. 3). There was significant relationship between intensity of the walk load and increment in the plasma hypoxanthine concentration with correlation coefficient of 0.556 as shown in Fig. 4. The serum

TABLE 1. *Changes in serum hypoxanthine concentration by intensity of walk load*

Intensity of the walk load	Serum concentration of hypoxanthine (μmol/liter)		
	Before load	10 minutes after load	difference
Mild (41-60%)	1.22±1.54	2.49±2.55**	1.27±2.09
Moderate (61-80%)	2.62±2.07	8.38±8.38**	5.75±5.17
High (81%-)	3.03±1.78	17.55±14.63**	14.57±14.14

**$p<0.01$ vs. before load

hypoxanthine levels were increased significantly by the walk load even at mild intensity between 40 and 60% with mean increment of 1.27±2.09 μmol/liter, at moderate intensity between 60 and 80% with mean increment of 5.75±5.17 μmol/liter, and at strenuous intensity more than 80% with mean increment of 14.57±14.14 μmol/liter (Table 1).

As shown in Fig. 5, there was also significant correlation between increment in the serum hypoxanthine concentration and PCI with correlation coefficient of 0.338 ($p<0.01$).

DISCUSSION

Hypoxanthine is produced in muscles, and its production is pronounced in the presence of hypoxia or following intense exercise, resulting in elevation of its serum levels. According to Bangsbo et al. (1992) and Hellsten-Westing et al. (1989), the serum levels of hypoxanthine increased to 5 folds of the resting levels after arm cranking and more than 10 folds after short distance maximum running. Urinary excretion of hypoxanthine also increases one hour after completion of exercise or during active daytime (Harkness et al. 1983).

A part of intracellular hypoxanthine is converted to IMP, and plasma hypoxanthine is metabolized to uric acid in the liver (Hellsten-Westing 1994). Previous studies demonstrated that plasma levels of hypoxanthine showed a peak at 10 to 20 minutes after completion of exercise (Harkness et al. 1983; Hellsten-Westing et al. 1989; Tullson et al. 1995). This study also demonstrated that the walk load at high intensity increased the serum hypoxanthine levels to more than 6 times of the resting values, showing a peak at 10 to 20 minutes after completion of it. Further, the serum hypoxanthine levels at 10 minutes after completion of the walk load were increased according to increase in intensity of exercise, and the serum hypoxanthine levels significantly increased even by mild and moderate intensity of walk load.

There are several indicators for intensity of exercise. In this study, heart rate response was used to estimate intensity of exercise. Generally, linear relationship is found between heart rate and oxygen consumption for various intensities of light to moderately heavy exercise, and the maximum oxygen consumption predicted from submaximal heart rate is generally within 10 to 20% of the

person's actual value (McArdle et al. 1991).

Changes in the serum levels of hypoxanthine are considered as a reflection on intracellular energy metabolism. Previous studies demonstrated that prolonged exercise and intermittent training at high intensity caused decrease in muscle ATP and increase in plasma hypoxanthine (Broberg and Sahlin 1989; Hellsten-Westing et al. 1993b). It was further shown that muscle contents of creatine phosphate and ATP are decreased even by mild intensity exercise (Green et al. 1983; McCully et al. 1988; Sahlin and Katz 1989). These results suggest that the serum hypoxanthine levels may be increased even by mild exercise as a reflection of these reactions in the muscle.

In this study, we compared serum hypoxanthine levels just before exercise and 10 minutes after completion of the walk load. Though the difference was small at mild to moderate intensity in the present study, there was statistically significant difference.

Ketai et al. (1987) reported that steady state exercise at 52, 76, and 97% of ventilatory threshold, and exercise at subventilatory threshold intensity (74% of ventilatory threshold) for a prolonged time period did not elevate the hypoxanthine levels above resting values. However, plasma hypoxanthine rose significantly after exercise at 124% of ventilatory threshold, and the peak plasma hypoxanthine levels after the maximum exercise were significantly greater than the resting levels. They concluded that elevation of the plasma hypoxanthine levels occurs during exercise at intensity that exceeds the ventilatory threshold. Duration and intensity of exercise in their study were almost same as those in our study. However, they did not measure plasma concentration of hypoxanthine just before exercise, but compared the post exercise values to the values at rest which were analyzed on the other days.

According to Stathis et al. (1994), muscle hypoxanthine is increased extensively after exercise and its increase is lower in the trained subjects. Furthermore, physical training suppresses the increase in the plasma levels of hypoxanthine after exercise (Hellsten-Westing et al. 1993b). These results suggest that discrepancy between resuts obtained by Ketai et al. (1987) and us may be due to difference in enduarance of the participants in the both studies.

PCI is calculated dividing the increment of heart rate during the walk load by walking speed (Steven et al. 1983), and decreases according to increase in energy efficiency during exercise. In this study, change in the serum levels of hypoxanthine showed significant positive correlation with physiological cost index, which suggests that change in the serum levels of hypoxanthine may be an indicator of efficiency of energy metabolism in the muscle during mild to high intensity of exercise.

Results in this study demonstrated that mild intensity exercise increases the serum levels of hypoxanthine, and suggested that change in them may be an extracellular metabolite monitoring the intracellular energy metabolism in the

muscle. Further study is necessary to elucidate if subjects with low physical endurance show increase in the serum levels of hypoxanthine by exercise at lower level of intensity, which was demonstrated in this study.

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