Changes in Serum Hypoxanthine Levels by Exercise in Obese Subjects

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To study on effect of obesity on changes in serum hypoxanthine with exercise, exercise stress testing with treadmill was performed on 7 obese subjects (body mass index [BMI], 30.6 ± 3.2 kg/m^2) and 16 healthy volunteers (BMI, 21.5 ± 2.10 kg/m^2). Expiratory gas analysis during exercise showed that peak Vo_2 was significantly lower in the obese group than in the control group (28.1 ± 4.0 *v* 37.1 ± 4.7 mL/kg/min; *P* < .001). Furthermore, the obese group had lower anaerobic threshold (AT) values (*P* < .005), respiratory quotient at AT (*P* = .003), and exercise capacity reserve (*P* = .002) than the control group. Baseline serum hypoxanthine levels were significantly higher in the obese group than in the control group (3.46 ± 3.70 *v* 1.23 ± 1.16 μmol/L; *P* < .05). Exercise induced a pronounced increase in serum hypoxanthine level in the obese group compared with the control group (10.65 ± 6.81 *v* 43.86 ± 4.56 μmol/L; *P* < .01). Serum levels of uric acid before and after load were also higher in the obese group than in the control group (404 ± 43 *v* 302 ± 77 μmol/L; *P* < .005). A pronounced increase in hypoxanthine with exercise may result in organ damage caused by free radicals, and intermittent training from mild intensity may be less hazardous for exercise treatment of obesity.

CONSUMPTION OF MUSCLE adenosine triphosphate (ATP) is pronounced with intense exercise or ischemia and is followed by formation of hypoxanthine via adenosine monophosphate (AMP) and inosine monophosphate (IMP). Hypoxanthine diffuses slowly from muscle to bloodstream, peaking 10 to 20 minutes after completion of exercise,[1] and 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 intracellular energy metabolism,[2] an indicator of tissue hypoxia,[3-5] and a marker of free radical formation after reperfusion.[6] Many studies have shown that hypoxanthine is produced in muscles according to intensity of exercise, and there is great individual variation of changes in plasma concentrations of hypoxanthine caused by intense exercise.[6-10] It has been reported that increases in serum hypoxanthine with exercise are more pronounced in sedentary subjects and in the fasting state[11] and less pronounced in trained subjects.[12,13] It has also been reported that muscle IMP accumulation with exercise is attenuated by carbohydrate supplementation.[14]

Changes in plasma hypoxanthine with exercise may be an important indicator for understanding metabolic changes induced by exercise in pathologic as well as physiologic conditions. Obesity is often accompanied by metabolic derangement, including hyperuricemia, which may lead to a pronounced increase in hypoxanthine and free radical formation with exercise. The present study was undertaken to study the effect of obesity on changes in serum hypoxanthine levels with exercise.

SUBJECTS AND METHODS

Subjects and Exercise Testing

Twenty-three subjects participated in the study; 7 obese subjects with mean age 25.0 ± 10.7 years and mean body mass index (BMI) 30.6 ± 3.2 kg/m^2 and 16 healthy volunteers with mean age 22.9 ± 6.3 years and mean BMI 21.5 ± 2.0 kg/m^2 (Table 1). They performed exercise stress testing on a treadmill according to the St Marianna Hospital protocol[15] 2 to 3 hours after food intake. Intensity of exercise was calculated by Karvonen's method using heart rate as an indicator.[16] Expiratory gas analysis during exercise was performed using the Hitachi 2900 Metabolic Measurement System (Nihon Koden, Tokyo, Japan). The expiratory gas was analyzed breath by breath for measurements of respiratory peak oxygen consumption (peak Vo_2). Anaerobic thresold (AT) was determined using the V-slope method.[7] Exercise capacity reserve (ECR) was calculated by subtracting oxygen consumption at AT from peak Vo_2.

Blood Sampling

Venous blood samples for chemical analysis were drawn from the anticubital vein before and at 10 minutes after secession of treadmill exercise. Blood was centrifuged immediately, and serum was removed and stored at −75°C until it was assayed.

Analytical Method

Oxypurinol was added to each sample as an internal standard before analysis. Twenty micromoliters of standard solution or serum was applied to high-performance liquid chromatography and analyzed by a modified method of Wung and Howell[18] as described by Hellsten-Westing et al[12] using a Gilson-305 equipped with Gilson (Middleton, WI) UV Master-1001 and system work station model MS-712. A SynChropak RP-P-100 column (250 × 2.1 mm internal diameter [ID], 100Å; Micra Scientific, Northbrook, IL) was used as a separation column, and SynChropak RP-P-300 (50 × 4.6 mm ID, 300Å; Micra Scientific) as a guard column. Hypoxanthine was separated under isocratic condition with the phosphate buffer as eluent, at a flow rate of 0.25 mL/min. A standard curve was made for 3.7 to 18.5 μmol/L of hypoxanthine, and the correlation coefficient of the area ratio to oxypurinol and concentration of hypoxanthine was 0.999.

Statistical Analysis

All data were presented as means ± SD. Differences between means for the groups were analyzed using 1-way analysis of variance (ANOVA) and Mann-Whitney *U* test, a level of $P < .05$ was considered statistically significant.

RESULTS

The obese group performed treadmill exercise for a shorter period than the control group (11.2 ± 1.2 *v* 14.5 ± 2.5 minutes;

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Table 1. Physical Characteristics of Subjects

Group	n	Age (yr)	Body Weight (kg)	BMI (kg/m^2)
Control	16	22.9 ± 6.3	63.3 ± 8.5	21.5 ± 2.0
Obese	7	25.0 ± 10.7	87.2 ± 20.3*	30.6 ± 3.2*

*$P < .001$ *v* control.

$P < .005$), and intensity of exercise calculated by Karvonnen's method was similar in both groups (73.1% ± 10.7% *v* 72.6% ± 11.1%). Peak Vo_2 was significantly lower in the obese group than in the control group (28.1 ± 4.0 *v* 37.1 ± 4.7 mL/kg/min; $P < .001$). Furthermore, the obese group had lower AT values of ($P < .005$), respiratory quotient at AT (AT-QR; $P = .003$), and exercise capacity reserve (ECR; $P = .02$) than the control group (Table 2). Baseline serum hypoxanthine was significantly higher in the obese group than in the control group (3.46 ± 3.70 *v* 1.23 ± 1.16 μmol/L; $P < .05$). Baseline of serum uric acid level was also higher in the obese group than in the control group (404 ± 43 *v* 302 ± 77 μmol/L; $P < .005$).

Treadmill exercise induced significant increases in serum levels of hypoxanthine and uric acid in both groups, as shown in Fig 1. Serum hypoxanthine level 10 minutes after exercise was higher in the obese group than in the control group (10.65 ± 6.81 *v* 3.86 ± 4.58 μmol/L; $P < .05$). Serum uric acid level increased to 440 ± 43 and 304 ± 67 μmol/L in the obese and control groups, respectively ($P < .002$). The change in hypoxanthine level in the obese group was 2.7 times the change in the control group.

DISCUSSION

This study shows for the first time that both serum hypoxanthine level at rest and uric acid level are increased in obesity. Although the duration of treadmill exercise was shorter and peak Vo_2 was lower in the obese group, and intensity of exercise was similar in both groups, the increase in serum hypoxanthine level was greater in the obese group (Table 2).

Serum levels of hypoxanthine are elevated in the presence of hypoxia or ischemia and after intense exercise. They are extracellular metabolites monitoring intracellular energy metabolism,[2] an indicator of tissue hypoxia.[3-5] Further, free radical is produced in the process to metabolize hypoxanthine to uric acid.[6] A pronounced increase in hypoxanthine with exercise in obesity, which was demonstrated in this study, may lead to organ damage because of free radical.

Obesity itself is a risk factor for atherosclerosis and often is accompanied by other risk factors such as glucose intolerance, hyperlipidemia, and hypertension. As a result, the incidence of coronary heart diseases and stroke is higher in obese subjects.[19] Diet and exercise therapy are essential in the treatment of obesity. However, intense exercise should be limited in obese patients with coronary disease. Further, this study suggests that intense exercise may be hazardous for obese subjects in a sense of free radical damage.

During intense exercise, hypoxanthine is produced in muscle cells through IMP, with a similar decrease in the total adenine nucleotide content in muscle.[21,22] The rate of elimination of adenine nucleotides from cells was markedly elevated with increasing intensity of exercise and moderately affected by duration of exercise.[23] Hypoxanthine is released slowly into the blood stream[8,9] and taken up by the liver, where it is converted to uric acid.[24] It was reported that plasma hypoxanthine levels peak 10 to 20 minutes after completion of intense exercise.[1] Ketai et al[7] report that steady-state exercise at subventilatory threshold intensity did not elevate hypoxanthine levels, but exercise at 124% of ventilatory threshold did. They conclude that elevation of the plasma hypoxanthine levels occurs during exercise at an intensity that exceeds the ventilatory threshold. Many other studies[6,8,10,21] have also demonstrated significant

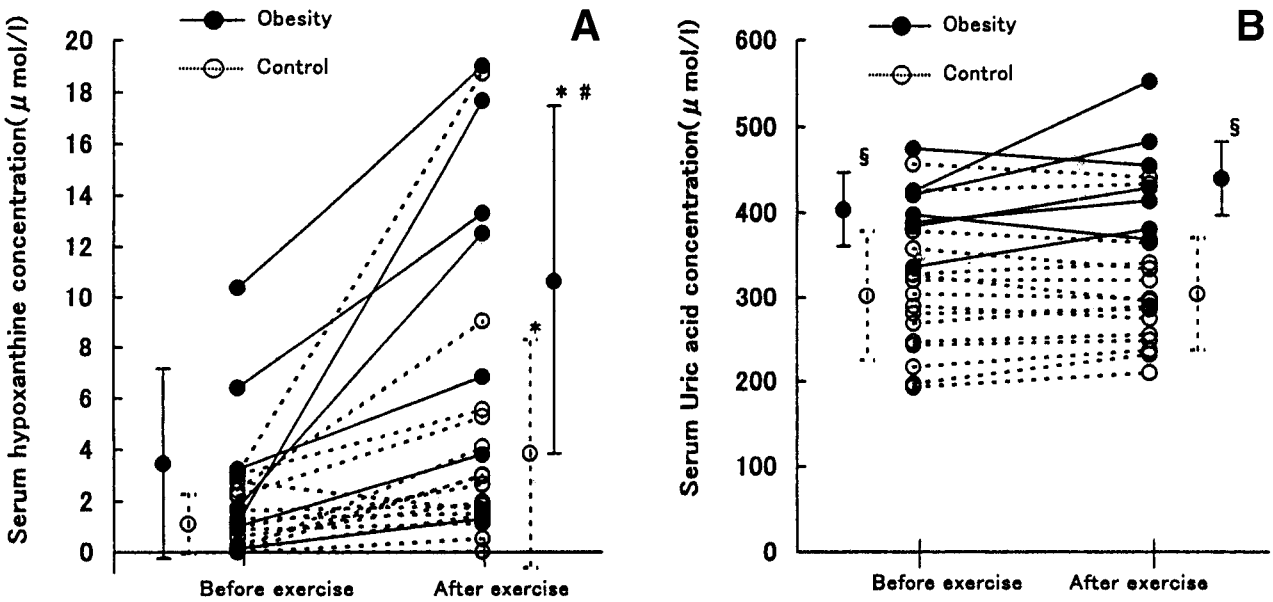

Fig 1. Changes in (A) serum hypoxanthine and (B) uric acid concentration before and after treadmill exercise in obese patients (●, n = 7) and the control group (○, n = 16). Values are means ± SD. *$P < .05$ *v* before exercise; #$P < .05$ *v* control; §$P < .005$ *v* control.

Table 2. Exercise Duration and Expiratory Gas Analysis During Treadmill Exercise

Group	Duration of Exercise (min)	Exercise Intensity (%)	Peak Vo_2 (mL/kg/min)	AT (mL/kg/min)	AT-RQ	ECR (mL/kg/min)
Control	14.5 ± 2.5	72.6 ± 11.1	37.1 ± 4.7	20.5 ± 2.6	0.83 ± 0.04	16.8 ± 3.6
Obese	11.2 ± 1.2*	73.1 ± 10.7	28.1 ± 4.0†	16.3 ± 2.4*	0.77 ± 0.09*	10.1 ± 3.4‡

*$P < .005$ *v* control.
†$P < .001$ *v* control.
‡$P < .02$ *v* control.

increases in serum hypoxanthine 10 minutes after completion of steady-state exercise at moderate to maximal intensity. According to Yamanaka et al,[20] exercise by ramp load at intensity exceeding AT also induces an increase in plasma hypoxanthine level, with a peak 10 minutes after exercise, which was also demonstrated in the control and obesity groups in this study.

This study also shows that serum levels of uric acid did not decrease but tended to increase in the obese group, as in case of hypoxanthine, suggesting that pronounced elevation of hypoxanthine by exercise in the obese group is caused by an increase in hypoxanthine formation by exercise.

Hellsten et al[9] demonstrated that sprint training for 6 weeks decreased the activity of adenosine-5′-phosphate deaminase and increased the activities of hypoxanthine-guanine phosphoribosyltransferase and phosphofructokinase in muscle. It has been also reported that high-intensity intermittent training causes a decrease in resting levels of muscle adenine nucleotides and prevents further loss of adenine nucleotides by intense exercise.[9,13,25] Further, Spencer et al[14] showed attenuated IMP accumulation in muscle with exercise during carbohydrate supplementation. We also have reported that serum hypoxanthine increases more extensively in the fasted than in the fed state and in sedentary than in physically active subjects.[10,11] These results suggest that increases in serum hypoxanthine levels are attenuated in trained subjects and when carbohydrate metabolism is dominant.

Compared with the control group, the obese group showed decreased AT and AT-RQ, suggesting that physical work capacity is lower and lipid metabolism is the dominant substrate for energy metabolism in the obese group. These factors may contribute to the pronounced increase in serum hypoxanthine levels with exercise.

For exercise treatment of obesity, intermittent training starting from mild intensity may be less hazardous, preventing pronounced increases in hypoxanthine; this conclusion requires further study.

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