Altered Purine Nucleotide Degradation During Exercise in Patients With Essential Hypertension

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Purine degradation occurs during strenuous muscle exercise and plasma levels of hypoxanthine (HX), purine degradation intermediate, increase. Purine nucleotide degradation has not been investigated in patients with essential hypertension (HTN). The present study determined whether purine nucleotide degradation is altered in patients with HTN. Cardiopulmonary exercise test was performed with serial measurements in blood lactate and plasma HX in 24 patients (14 men and 10 women) with essential HTN (World Health Organization [WHO] class I to II; mean age, 57.7 ± 2.1 years) and 24 age-, sex-matched normal subjects. Exercise was terminated either by severe fatigue or excess blood pressure increase. Peak work rate (WR) (normal *v* HTN, 151 ± 10 *v* 135 ± 8 W, not significant [NS]) was not different, but peak oxygen uptake (peak V_{O_2}, 26.3 ± 1.5 *v* 22.2 ± 0.9 mL/min/kg, $P < .05$) and anaerobic threshold were lower in patients with HTN. Resting levels of blood lactate and plasma HX were similar, but the increment from rest to peak exercise (Δ) for lactate (Δlactate: 4.4 ± 0.4 *v* 3.4 ± 0.4 mmol/L, $P < .05$) and for HX (ΔHX, 15.9 ± 2.2 *v* 9.1 ± 1.1 μmol/L, $P < .05$) were significantly smaller in patients with HTN. When normalized by the peak WR, ΔHX/peak WR (0.105 ± 0.013 *v* 0.069 ± 0.007 μmol/L/W, $P < .05$) was significantly lower in patients with HTN. Patients with HTN exhibited reduced HX response to exercise with impaired exercise capacity. The exercise-induced changes in plasma HX were smaller in patients with HT when normalized with peak WR. These results suggest that the purine nucleotide degradation is reduced in patients with HTN.

STRENUOUS MUSCLE exercise causes rapid consumption of adenosine 5′-triphosphate (ATP) and increase in adenosine 5′-diphosphate (ADP) and adenosine 5′-monophosphate (AMP).[1,2] Purine nucleotide degradation is accelerated to produce inosine 5′-monophosphate (IMP), hypoxanthine (HX), and in some tissues, xanthine and uric acid[3-5] (Fig 1). HX is a main metabolite in the skeletal muscle, which subsequently appears in the plasma. Thus, the level of plasma HX can be an index of purine nucleotide degradation during exercise. Because the production of purine metabolites are a consequence of the ATP consumption and AMP accumulation,[6] they are considered to be markers for what is called "cell energy crisis"[7] and are thought to reflect energy depletion in skeletal muscle.

We have previously studied purine metabolism in heart failure patients with impaired exercise tolerance and found that purine degradation when normalized with exercise work rate (WR) was reduced in these patients.[8] Patients with hypertension (HTN) also reported to have exercise intolerance.[9] Based on our previous study, we hypothesized that patients with HTN are more intolerant of energy depletion during exercise, which may contribute to the reduced exercise capacity in hypertensives. However, to our knowledge, purine metabolism during dynamic exercise has not been evaluated in patients with HTN. To determine whether purine degradation during exercise is altered in HTN, we measured plasma levels of HX and blood lactate concentration during cardiopulmonary exercise testing in patients with essential HTN and compared these data with those of age- and sex-matched normal control subjects.

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SUBJECTS AND METHODS

Subjects

We studied 24 patients (14 men and 10 women) with essential HTN (World Health Organization [WHO] class I to II; mean age ± SEM: 57.7 ± 2.1 years) and 24 age-, and sex-matched normal subjects (14 men and 10 women, 57.1 ± 2.0 years). Body mass index (BMI) in patients with HTN was 23.4 ± 0.5 kg/m^2 and in normal subjects 23.1 ± 0.4 kg/m^2. History, physical examination, electrocardiogram (ECG), chest x-ray, and echocardiogram were performed to exclude any cardiovascular disease in these 24 normal control subjects. Patients with HTN met the following criteria during the run-in period: diastolic blood pressure (DBP) in the range 90 to 110 mmHg and/or systolic blood pressure (SBP) in the range 160 to 200 mmHg. Secondary HTN was excluded by clinical examination, routine blood and urine analysis, hormonal examinations, and in some cases, by renogram, and the diagnosis of uncomplicated essential HTN was established. All patients had not been treated previously for HTN. The prevalence of smokers in the 2 groups (normal *v* HTN, 33% *v* 38%, not significant [NS]) was not significantly different. Cardiothoracic ratio (46.5 ± 1.0 *v* 50.2% ± 0.9%, $P < .05$) in chest x-ray was significantly larger in patients with HTN and left ventricular ejection fraction (69.7 ± 1.8 *v* 62.6% ± 2.9%, NS) assessed by echocardiogram was not significantly different between the 2 groups.

To determine the blood lactate and plasma HX response to exercise in subjects with a wide range of exercise capacity, an additional 41 normal subjects (mean age, 51.0 ± 1.8 years, 30 men and 11 women) with varying degrees of exercise tolerance (average peak WR, 202.9 ± 11.7 W; range, 55 to 403 W) were also included in the study. The study protocol was approved by the Ethics Committee of Tottori University, and all subjects gave written informed consent to perform the protocol.

Exercise Test

Cardiopulmonary exercise test was performed using an upright bicycle ergometer (with ramp protocol) as previously described.[10] Briefly, after a 4-minute unloading cycling, the exercise load was increased by incremental loading of 10 or 20 W/min. The criteria for terminating exercise were: (1) severe fatigue or (2) excess BP increase;

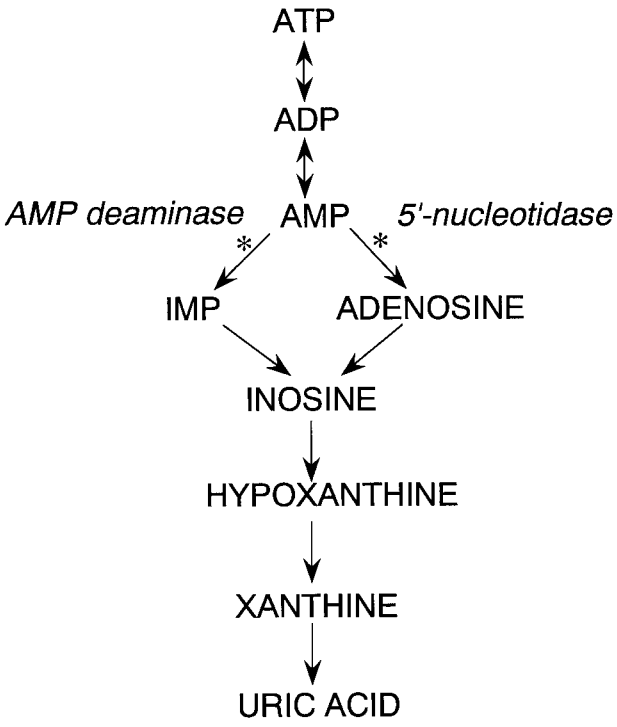

Fig 1. Purine metabolism in the skeletal muscle. ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; AMP, adenosine 5′-monophosphate; IMP, inosine 5′-monophosphate.

SBP greater than 260 mmHg and/or DBP greater than 130 mmHg. Heart rate and ECG were monitored continuously, and BP was measured every minute during and after exercise by cuff technique. Oxygen uptake (V_{O_2}), carbon dioxide output, and minute ventilation were measured at rest and throughout the exercise period using a 280E Aeromonitor (Minato Medical Science, Osaka, Japan). Anaerobic threshold was determined mainly by the V-slope method.[11,12] Peak V_{O_2} was defined as the maximal V_{O_2} attained during exercise. Peak respiratory exchange rate was calculated by dividing carbon dioxide output by V_{O_2} at peak exercise.

Determination of Blood Samples

A short polyethylene cannula was placed in a forearm vein for the blood sampling. Blood samples for lactate were obtained at rest, immediately after exercise, 3, 5, 10, 15, 20, and 30 minutes postexercise. Blood lactate was determined by enzymatic method using lactate analyzer (model 23L; YSI, Yellow Springs, OH). Blood specimens for plasma HX were obtained at rest, immediately after exercise, and 10, 20, and 30 minutes postexercise. Plasma HX concentrations were measured by high performance liquid chromatography as described previously.[13] The increment in blood lactate or plasma HX levels from rest to peak exercise (Δlactate or ΔHX) was divided by the peak WR and the normalized ratio was defined as follows: normalized blood lactate ratio = (peak blood lactate − rest blood lactate)/peak WR and normalized plasma HX ratio = (peak plasma HX − rest plasma HX)/peak WR.

Statistical Analysis

Statistical analysis for the comparisons of cardiac and ventilatory variables between the 2 groups were performed by Mann-Whitney *U* test. Repeated measures analysis of variance (ANOVA) was used to examine the difference in blood lactate and plasma HX responses to exercise. When 3 groups were compared, ANOVA followed by Fisher's range test was applied. All analyses were performed with a StatView statistical program (Version 5.0, SAS Institute, Cary, NC). The differences were considered significant when *P* values were less than .05. Statistical values are presented as mean ± SEM.

RESULTS

Cardiopulmonary Exercise Data

All normal subjects stopped exercise with severe fatigue. Of 24 hypertensives, 17 patients stopped exercise with severe fatigue, and 7 stopped exercise because of excess BP increase during exercise. Peak WR (normal *v* HTN, 151 ± 10 *v* 135 ± 8 W, NS) was not significantly different between the 2 groups. Peak V_{O_2} (26.3 ± 1.5 *v* 22.2 ± 0.9 mL/min/kg, $P < .05$) and anaerobic threshold (15.7 ± 1.0 *v* 12.9 ± 0.5 mL/min/kg, $P < .05$) were significantly lower in patients with HTN compared with normal subjects. Peak respiratory exchange ratio (1.18 ± 0.02 *v* 1.13 ± 0.02, NS) was not significantly different between the 2 groups. Heart rate at rest (70 ± 2 *v* 73 ± 3 beats/min, NS) and at peak exercise (151 ± 4 *v* 151 ± 5 beats/min, NS) were not significantly different, but resting SBP (132 ± 3 *v* 185 ± 4 mmHg, $P < .01$) and resting DBP (80 ± 2 *v* 101 ± 2 mmHg, $P < .01$), peak SBP (208 ± 6 *v* 234 ± 6 mmHg, $P < .01$), and peak DBP (97 ± 3 *v* 111 ± 3 mmHg, $P < .01$) were significantly higher in patients with HTN.

Blood Lactate and Plasma HX Response to Exercise

Resting levels of blood lactate (normal *v* HTN: 0.67 ± 0.03 *v* 0.73 ± 0.04 mmol/L, NS) and plasma HX (3.7 ± 0.7 *v* 3.5 ± 0.6 μmol/L, NS) were similar between normal subjects and patients with HTN. Blood lactate and plasma HX levels significantly increased after exercise in both groups. Blood lactate response to exercise was significantly reduced in patients with HTN (ANOVA, $P = .033$) (Fig 2A). Plasma HX response to exercise was also significantly reduced in patients with HTN (ANOVA $P < .0001$) (Fig 2B). The increment in lactate (Δlactate, 4.4 ± 0.4 *v* 3.4 ± 0.4 mmol/L, $P < .05$) and the increment in HX (ΔHX, 15.9 ± 2.2 *v* 9.1 ± 1.1 μmol/L, $P < .05$) from rest to peak exercise were significantly smaller in patients with HTN. Because the degrees of purine nucleotide degradation during exercise are related to the amount and duration of the power output,[14,15] changes in blood lactate and plasma HX were normalized with the peak WR. The ratio for Δlactate to peak WR (Δlactate/peak WR) was not significantly different (Fig 2C), but the ratio for ΔHX to peak WR (ΔHX/peak WR) was significantly smaller in patients with HT compared with normal subjects (Fig 2D).

To further evaluate the effects of exercise termination on purine degradation, patients with HTN were divided into 2 subgroups based on the reasons of exercise termination. We compared exercise and metabolic variables among normal controls (n = 24), patients whose end point was excess BP increase (BP group, n = 7) and patients whose end point was fatigue (fatigue group, n = 17). The results are summarized in Table 1. Peak WR was not significantly different among groups, but anaerobic threshold was significantly smaller in both hypertensive groups. ΔPlasma HX values were significantly reduced in patients with both fatigue and BP groups compared with that of

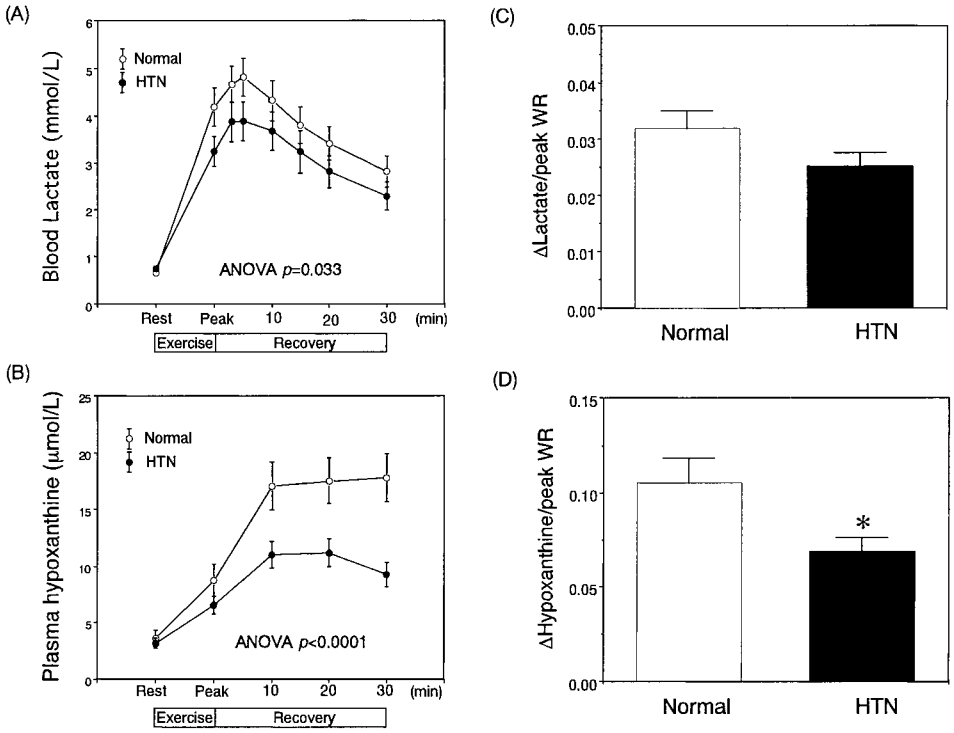

Fig 2. Blood lactate (A) and plasma hypoxanthine (B) responses to exercise in normal subjects and in patients with essential HTN. Bar graphs showing changes in blood lactate normalized by peak work rate (Δlactate/peak WR) (C) and changes in plasma HX normalized by peak work rate (ΔHX/peak WR) (D) in normal subjects and in patients with HTN. Values are mean ± SEM. *$P < .05$ *v* normal subjects.

normal controls. The value of ΔHX/peak WR (0.105 ± 0.013, 0.075 ± 0.009, and 0.055 ± 0.011 μmol/L/W) became gradually smaller from normal control to the BP group, and the BP group had a significantly smaller ratio compared with that of normal subjects.

In an additional 41 subjects, the ratios of metabolic changes per peak WR were 0.105 ± 0.007 μmol/L/W for ΔHX and 0.030 ± 0.001 mmol/L/W for Δblood lactate (graphs not shown). These data showed that the normal subjects with various exercise capacity have relatively constant ratios for plasma HX and blood lactate when normalized with peak WR. We also evaluated the metabolic response based on the smoking status and found that lactate production and purine degradation did not differ significantly between smokers and nonsmokers in both the control and hypertensive groups.

DISCUSSION

Our study found that plasma HX response to exercise was reduced in patients with essential HTN compared with age-, sex-matched normal subjects. The reduced purine degradation during exercise in hypertensives is based on the fact that both absolute values and the increment in plasma HX normalized

Table 1. Cardiopulmonary Exercise Data and Metabolic Variables in Normal Subjects and Subgroups of Hypertensives Stratified With Reasons for Exercise Termination

		HTN	
	Normal Subjects (n = 24)	Fatigue Group (n = 17)	BP Group (n = 7)
Cardiopulmonary exercise variables			
Peak WR (W)	151 ± 10	138 ± 8	127 ± 19
Anaerobic threshold (mL/min/kg)	15.7 ± 1.0	12.9 ± 0.6*	12.7 ± 0.9*
Peak Vo_2 (mL/min/kg)	26.3 ± 1.5	22.7 ± 1.1	20.9 ± 1.5
Peak respiratory exchange ratio	1.18 ± 0.02	1.13 ± 0.02	1.13 ± 0.02
Metabolic variables			
ΔLactate (mmol/L)	4.4 ± 0.4	3.8 ± 0.5	2.3 ± 0.4*
ΔHypoxanthine (μmol/L)	15.9 ± 2.2	10.8 ± 1.4*	6.6 ± 1.4*
ΔLactate/peak WR (mmol/L/W)	0.032 ± 0.003	0.028 ± 0.003	0.019 ± 0.003
ΔHypoxanthine/peak WR (μmol/L/W)	0.105 ± 0.013	0.075 ± 0.009	0.055 ± 0.011*

NOTE. Values are mean ± SEM.

Abbreviations: HT, hypertension; BP group, hypertensive patients whose exercise end point was excess BP increase; fatigue group, hypertensive patients whose exercise end point was severe fatigue; WR, work rate; Vo_2, oxygen uptake; Δ, the increments from resting value to maximal value.

* $P < .05$ *v* normal subjects.

with the peak WR (ΔHX/peak WR) were significantly smaller in patients with HTN.

Purine Degradation During Exercise in Hypertensives

In the present study, patients with HTN had reduced peak V_{O_2} and anaerobic threshold compared with normal controls, suggesting that maximal exercise capacity and aerobic capacity were reduced in hypertensives. Despite the impaired exercise tolerance, BP at peak exercise was significantly higher in patients with HTN. Serial measurements of plasma HX concentrations showed that, compared with the age-, sex-matched normal controls, the absolute plasma HX levels after exercise was smaller in hypertensives. Furthermore, we showed that the workload-adjusted plasma HX response, but not blood lactate response, was reduced in patients with HTN. These results suggested that the purine nucleotide degradation during exercise is significantly altered in patients with essential HTN.

Mechanisms of Altered Purine Degradation in Hypertensives

The present study did not clarify the precise mechanism(s) responsible for the reduced purine degradation in HTN, but the following factors should be considered. First, smaller exercise workload in hypertensives may be responsible. Subgroup of patients who stopped exercise by excess BP increase showed smaller purine degradation. Therefore, the reduced purine degradation may be attributed to the less severe fatigue in patients with HTN. However, the workload-adjusted purine metabolite was significantly smaller in hypertensives, suggesting that the reduced purine degradation cannot totally be explained by the difference in exercise capacity. Second, purine degradation occurs in exercising skeletal muscle,[1,2] therefore, the difference in muscle volume may be a factor. Although we cannot completely exclude this possibility, we believe that it is unlikely, because purine degradation was compared between the closely matched 2 groups with similar BMI. Finally, decreased activity of AMP deaminase, the enzyme responsible for purine degradation,[1,2] may explain the observed difference, as has been suggested for patients with heart failure.[8] In patients with heart failure, plasma adenosine levels are elevated according to the disease severity[16] with an increase in 5′-nucleotidase activity, the enzyme responsible for adenosine production.[17] If these changes occurred in HTN, the decreased purine nucleotide degradation during exercise could be explained by the shunting of the catabolic pathway from AMP-IMP toward adenosine production, although the present study does not address these issues.

Clinical Implications

What is the clinical implication of the reduced purine degradation during exercise in HTN? It is reported that blood and urine uric acid and its precursors (inosine and HX) increase during exercise in patients with glycogen storage disease, and that ATP consumption and AMP accumulation are responsible for the overproduction of these purine metabolites.[6] Therefore, purine metabolites are considered to be markers for what we called "cell energy crisis"[7] and reflect energy depletion in skeletal muscle. In the present study, we noted that peak plasma HX levels after exercise were reduced in patients with HTN. These results suggest that the maximal energy depletion in skeletal muscle may be smaller at peak exercise in patients with HTN. The mechanisms of exercise intolerance in hypertensives are multifactorial and several factors, including diastolic dysfunction of the heart, high peripheral vascular resistance, and impaired vasodilatory capacity, are considered to be important.[9] The findings of the present study suggested that patients with HTN may be more intolerant of energy depletion during exercise, which may contribute to the reduced exercise capacity in patients with HTN.

Limitation to the Study

We used the normalized ratio of exercise plasma HX and blood lactate to evaluate the purine degradation and lactate production during exercise. However, questions remain about the validity of the workload-adjusted metabolic response. To validate the methodology, we measured peak levels of plasma HX and blood lactate during cardiopulmonary exercise testing in 41 normal control subjects with varying exercise capacity. The values of ΔHX/peak WR and Δblood lactate/peak WR were relatively constant over the wide range of exercise WR in these normal subjects. Thus, it seems reasonable to use the workload-adjusted purine degradation and lactate production for the data analysis.

CONCLUSIONS

In conclusion, patients with HTN exhibited reduced HX response to exercise with impaired exercise capacity. The exercise-induced changes in plasma HX were smaller in patients with HTN when normalized with peak WR. These results suggest that the purine nucleotide degradation is reduced in patients with HTN. Mechanisms of altered purine degradation in hypertensives and its relationship to the exercise intolerance need further investigation.

REFERENCES

1. Sahlin K, Palmskog G, Hultman E: Adenine nucleotide and IMP contents of the quadriceps muscle in man after exercise. Pflugers Arch 374:193-198, 1978

2. Norman B, Sollevi A, Kaijser L, et al: ATP breakdown products in human skeletal muscle during prolonged exercise to exhaustion. Clin Physiol 7:503-509, 1987

3. Fox IH: Metabolic basis for disorders of purine nucleotide degradation. Metabolism 30:616-634, 1981

4. Fox IH: Human ribonucleotide catabolism: Clinical and biochemical significance. Nutr Metab 16:65-78, 1974

5. Sutton JR, Toews CJ, Ward GR, et al: Purine metabolism during strenuous muscular exercise in man. Metabolism 29:254-260, 1980

6. Mineo I, Kono N, Hara N, et al: Myogenic hyperuricemia A common pathophysiologic feature of glycogenosis types II, V, and VII. N Engl J Med 317:75-80, 1987

7. Fox IH, Palella TD, Kelley WN: Hyperuricemia: A marker for cell energy crisis. N Engl J Med 317:111-112, 1987

8. Kinugawa T, Ogino K, Noguchi N, et al: Altered purine and glycogen metabolism in skeletal muscle during during exercise in patients with heart failure. Metabolism 48:484-488, 1999

9. Lim PO, MacFadyen RJ, Clarkson PBM, et al: Impaired exercise tolerance in hypertensive patients. Ann Intern Med 124:41-55, 1996

10. Kinugawa T, Ogino K, Noguchi N, et al: Sympathetic nervous response relative to the adenosine trophosphate supply-demand imbal-

ance during exercise is augmented in patients with heart failure. Chest 114:1295-1300, 1998

11. Sue DY, Wasserman K, Morrica RB, et al: Measurement of anaerobic threshold by V-slope method in patients with chronic obstructive lung disease and normal subjects. Chest 94:931-938, 1988

12. Beaver WL, Wasserman K, Whipp BJ: A new method for detecting the anaerobic threshold by gas exchange. J Appl Physiol 60:2020-2027, 1986

13. Hisatome I, Kotake H, Mashiba H: Determination of oxypurine and its clinical implication. Jpn J Clin Med 47:413-416, 1989

14. Ketai LH, Simon RH, Kreit JW, et al: Plasma hypoxanthine and exercise. Am Rev Respir Dis 136:98-101, 1987

15. Yamanaka H, Kawagoe Y, Taniguchi A, et al: Accelerated purine nucleotide degradation by anaerobic but not by aerobic ergometer muscle exercise. Metabolism 41:364-369, 1992

16. Funaya H, Kitakaze M, Node K, et al: Plasma adenosine levels increase in patients with chronic heart failure. Circulation 95:1363-1365, 1997

17. Funaya H, Sato H, Koretsune Y, et al: The cellular mechanisms by which plasma adenosine levels increase in patients with chronic heart failure. Circulation 96:I-267, 1997 (suppl 1, abstr)