Excessive Purine Degradation during Semi-ischemic Forearm Test in Patients with Diabetes Mellitus


Abstract

Objective The aim of this study was to investigate whether or not the purine degradation in the skeletal muscle during forearm exercise is augmented in patients with diabetes mellitus (DM).

Methods We used the semi-ischemic forearm test to examine the release of lactate (ALAC), ammonia (Amm) and hypoxanthine (BXH) before exercise, 0, 4, 10, and 60 minutes after exercise in eleven diabetic patients and seven normal controls.

Results The sum of the increased HX (DM vs Controls: 26.1±21.2 vs 7.8±5.9 μmol/l, p<0.05) was greater in diabetic patients. When patients were divided into the excessive response group (n=7) and normal response group (n=4), the maximum increments in ΔHX and ΔAm in the excessive response group (16.8±3.2 μmol/l and 122±60 μmol/l) were greater (p<0.05) than those in the control group (3.6±3.0 μmol/l and 32±34 μmol/l) and the normal response group (2.9±2.9 μmol/l and 27.4±12.7 μmol/l). ΔLAC both in the excessive response group (5.4±1.5 mmol/l) and the normal response group (3.6±1.0 mmol/l) were higher (p<0.05) than that of the control group (1.7±0.5 mmol/l). The prevalence of diabetic retinopathy was higher in the excessive response group (75% vs. 25%).

Conclusion These data suggest that patients with DM, especially with microangiopathy have augmented purine degradation during the semi-ischemic forearm test. Factors responsible for the augmented purine degradation in these patients remain to be determined. (Internal Medicine 42: 788–792, 2003)

Key words: hypoxanthine, lactate, ammonia, diabetes mellitus, purine degradation

Introduction

In skeletal muscle, adenosine triphosphate (ATP) is catalyzed to adenosine diphosphate (ADP) and adenosine monophosphate (AMP) during exercise (1). The cells try to maintain a high energy charge (EC) even when the concentration of ATP falls and the energy level decreases. The EC formula: EC = (ATP+0.5 ADP)/(ATP+ADP+AMP), indicates that a reduction in the concentration of AMP via activation of AMP deaminase keeps EC high during ATP deficiency (2).

Excessive release of hypoxanthine (HX) from the exercising muscle has been reported in patients with inherited (3–6) and acquired disorders (7–11). Based on the above concept, the excessive purine degradation in these diseases is due to activation of the purine nucleotide cycle, because AMP deaminase is activated by the reduction of the EC. Of interest, exercise tolerance in patients with these diseases is commonly limited. The reduction of exercise tolerance might be at least in part, related to the cell energy crisis in the exercising muscle. In patients with diabetes mellitus (DM), exercise tolerance is lower, and hypoperfusion in skeletal
Table 1. Characteristics of the Patients with Diabetes Mellitus

<table>
<thead>
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<th>Case No.</th>
<th>Age/Sex</th>
<th>Diagnosis</th>
<th>HbA1c (%)</th>
<th>Duration of DM (years)</th>
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<th>HL</th>
<th>Smoking</th>
<th>Obesity</th>
<th>Retinopathy</th>
<th>Nephropathy</th>
<th>Neuropathy</th>
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Mean±SD 9.4±2.9 11.4±4.5


Although it is reasonable to measure the difference between arterial and venous (A-V) concentration of the metabolites to examine muscles metabolism, we measured changes in the level of metabolites in venous samples. In a preliminary study, we compared the changes in A-V concentration difference of ΔHX, ΔAmm and ΔLAC in the semi-ischemic forearm with the changes in venous blood samples taken from the antecubital vein (n=5). The increases in A-V difference of ΔHX, ΔAmm and ΔLAC observed in the semi-ischemic forearm test were exactly the same as those in venous difference of ΔHX, ΔAmm and ΔLAC. Therefore, the changes in the level of metabolites in the venous sample seem to reflect the metabolism of the skeletal muscles at semi-ischemic handgrip test.

Results

Release of hypoxanthine from the exercising muscle

Figure 1 shows the changes in ΔHX from basal concentrations. Sum of the ΔHX after semi-ischemic exercise (ΣΔHX) was significantly (p<0.05) greater in diabetic subjects (26.1±21.2 μmol/l) compared to normal controls (7.8±5.9 μmol/l).

Release of ammonia and lactate from the exercising muscle

We subdivided the patients into the excessive response group and the normal response group according to whether the increment in HX exceeds mean+2 S.D. of normal controls or not. In seven diabetic patients (patient Nos. 1 to 7 in Table 1), plasma HX was increased above the mean+2 S.D.
of the control group at least one time point after the semi-ischemic forearm exercise. Figure 2 shows the $\Delta$HX, $\Delta$Amm and $\Delta$LAC in diabetic patients with excessive response, those with normal response, and normal controls. $\Delta$HX in the excessive response group was significantly greater compared to both the control group and the normal response group (Fig. 2A). The $\Delta$Amm in the excessive response group was significantly higher than that in either the control group or the normal response group (Fig. 2B). The time course of the decline of concentrations of LAC was different between the excessive response group and the normal response group. Namely, in the normal response group, the concentrations of LAC levels immediately returned to the baseline level, however, in the excessive response group, the LAC levels at 4 and 10 minutes postexercise were significantly higher than those in both the control and normal response groups (Fig. 2C).

**HX, Amm, and LAC in patients with or without diabetic retinopathy**

As shown in Fig. 3, $\Delta$HX at 10 minutes and $\Delta$LAC at 10 minutes after exercise were significantly ($p<0.05$) greater in patients with retinopathy (14.9±7.8 μmol/l and 1.7±1.0 mmol/l; respectively) than in patients without retinopathy (2.7±3.0 μmol/l and 0.2±0.05 mmol/l; respectively). $\Delta$LAC at 0 minute in patients with retinopathy and patients without retinopathy (4.9±1.9 mmol/l and 3.9±1.6 mmol/l; respectively) were not significantly different, but both were significantly ($p<0.01$ and $p<0.05$; respectively) greater than that of controls (1.8±0.6 mmol/l). At 0 and 4 minutes, $\Delta$Amm values of patients with retinopathy were higher than those of both control and patients without retinopathy but the differences did not reach to a significant level (data not shown).
It is well established that exercise increases plasma HX in some pathophysiological states, including glycogen storage disease types III, V, VII (3), mitochondrial myopathy (4), hypoparathyroidism (7), hyperthyroidism (8, 9), congestive heart failure (10), and chronic renal failure with hemodialysis (11). The increase in plasma HX is associated with concomitant increase in plasma Amm. These findings suggest that HX is a good index of purine degradation through the purine nucleotide cycle, because the increased AMP deamination via activation of AMP deaminase could accelerate the subsequent breakdown of inosine monophosphate to inosine, thereby leading to the increased formation of HX and Amm (1). Previous studies from our laboratory (9, 13) showed that both HX and Amm levels after forearm test increase in parallel. Thus, the augmented response in plasma HX to semi-handgrip exercise with the elevation of plasma Amm in the diabetic patients would reflect the activation of the purine nucleotide cycle in the exercising muscle.

The possible mechanisms of excessive purine degradation in the skeletal muscle after the semi-ischemic forearm test are: a) an “absolute” disturbance of the supply of ATP caused by deficient glycogenolysis or glycolysis; or b) a

“relative” disturbance of the supply of ATP, i.e., the demand for ATP exceeds its supply. When there was an absolute disturbance, there was no accompanying increase in blood LAC, but a relative disturbance was associated with an increase in blood LAC. In this study, an increase in plasma HX was accompanied by an increase in blood LAC. Thus, the excessive purine degradation in diabetic patients could be due to the result of a “relative” disturbance in the supply of ATP, i.e., when the demand for ATP exceeded its supply during exercise.

We investigated the factors responsible for the excess purine degradation in diabetic patients, however, age, mode of therapy, HbA1c levels (excess response vs. normal response: 9.6±3.6 vs 9.3±0.9 %, n.s.), duration of diabetes (12.4±4.2 vs 9.5±5.1 years, n.s.), prevalence of hyperlipidemia, hypertension, or obesity were not significantly different between the two groups. Thus, diabetic control, duration of DM, or presence of co-mobid conditions did not appear to influence the excess purine degradation in our patients. We did not evaluate CK levels during forearm test in this study, but we have previously reported that there is no change in CK concentrations during the forearm test (14). Therefore, it is unlikely that purine degradation is related to CK change during the semi-ischemic forearm test.

**Delayed decline in blood LAC after semi-ischemic handgrip test**

The decline of blood lactate after forearm exercise in the excessive response group was markedly slower than that in the normal response group. A similar phenomenon has also been observed both in patients with Fabry disease (5) and cyanotic heart disease (unpublished data) after the semi-ischemic forearm test, which resulted from the persistent peripheral hypoperfusion of the skeletal muscle after exercise. In addition, the slow decline of blood LAC was proportional to the relative ischemia of the exercising muscle after forearm test by means of thermography and near-infrared spectroscopy (15). Thus, we believe that blood LAC immediately after exercise reflects degree of anaerobic metabolism, and blood LAC at 10 minutes after exercise is influenced by washout of LAC with blood flow. We speculate that the persistent peripheral hypoperfusion after exercise may be important for the delayed decline in the blood LAC after exercise. Two factors are considered to be important for the persistent hypoperfusion of skeletal muscle in the diabetic patients. One is microvascular complication and other is endothelial dysfunction. Our patients with diabetic retinopathy had higher blood LAC levels at 10 minutes after forearm exercise test. In addition, reactive hyperemia after ischemia causes flow-mediated dilation via endothelial release of nitric oxide and this mechanism is impaired by the endothelial dysfunction. Diabetes is a well-known condition for the endothelial dysfunction. The delayed decline in blood LAC concentrations after exercise might be due to the impaired post-ischemic hyperemia as a result of endothelial dysfunction in DM.
Limitations to the study

This study has several limitations. First, we acknowledge that the study population was small in number. Small sample size may have contributed to the lack of statistical significance of some of the results, and did not allow us to perform multivariate analysis to detect factors responsible for the excess purine degradation. Second, number of control subjects was also small, but we would like to point out that control values for metabolites in this study were similar to those reported in our previous studies. Third, we acknowledge that the absence of clearance data for the metabolite is a limitation. Theoretically, plasma HX concentrations are raised by either increased production or decreased renal clearance of HX. Since we used the semi-ischemic forearm test to study the purine degradation in the present study, we could minimize the influence on the renal clearance of purine nucleotides with this type of exercise.

Conclusions

These data suggest that patients with DM, especially with microangiopathy have augmented purine degradation during the semi-ischemic forearm test. Factors responsible for the augmented purine degradation during semi-ischemic forearm test in these patients remain to be determined.

References