Excess Purine Degradation in Exercising Muscles of Patients with Glycogen Storage Disease Types V and VII

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Abstract

To investigate purine catabolism in exercising muscles of patients with muscle glycogen storage disease, we performed ischemic forearm exercise tests and quantitated metabolites appearing in cubital venous blood. Two patients with glycogen storage disease type V and three with glycogen storage disease type VII participated in this study.

Basal lactate concentrations lowered in every patient with glycogen storage disease type V or type VII. Two patients with glycogen storage disease type VII, who had markedly elevated concentrations of serum uric acid (14.3 and 11.9 mg/dl, respectively), showed high basal concentrations of ammonia (118 and 79 μmol/liter, respectively; 23±4 μmol/liter in healthy controls) and of hypoxanthine (23.4 and 20.4 μmol/liter, respectively; 2.0±0.4 μmol/liter in healthy controls). Other patients showed near normal measurements of these metabolites.

After forearm exercise, ammonia, inosine, and hypoxanthine levels increased greatly in every patient studied, in contrast with the lack of increase in lactate levels. The incremental area under the concentration curves for venous ammonia was 13-fold greater in the glycogen storage disease group than in controls (1,120±182 vs. 83±26 μmol · min/liter). The incremental areas of inosine and hypoxanthine were also greater in the glycogen storage disease group (29.2±7.2 vs. 0.4±0.1 and 134.6±23.1 vs. 14.9±3.2 μmol · min/liter, respectively). The incremental areas of ammonia in controls and in glycogen storage disease patients strongly correlated with those of hypoxanthine ($r = 0.984$, $n = 11$, $P < 0.005$).

These findings indicated that excess purine degradation occurred in the exercising muscles of patients with glycogen storage disease types V and VII, and suggested that the ATP pool in the exercising muscles may be deranged because of defective glycogenolysis or glycolysis.

Introduction

Glycogen storage disease (GSD)[1] type V (muscle phosphorylase deficiency or McArdle disease) (1) and type VII (phosphofructokinase deficiency or Tarui disease) (2) are muscle-energy diseases affecting glycogenolysis and glycolysis, respectively (3). Patients with these diseases have common muscular symptoms such as easy fatigability, stiffness, and pain on muscular

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1. *Abbreviation used in this paper:* GSD, glycogen storage disease.

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exertion when energy demands are increased. The symptoms are considered to be due to defective ATP generation in intramuscular glycolysis, which may lead to a critical shortage of intramuscular ATP. However, such ATP depletion has not been demonstrated in contractured muscles from patients with GSD type V (4, 5). On the other hand, exercised, cramped muscle from an animal model of defective muscle glycolysis showed marked depletion of ATP (6).

Vigorous exercise normally drives the catabolic reactions of adenine nucleotides in muscle and their metabolites appear in circulating blood (Fig. 1). Since 1928 it has been known that ammonia arises in muscle by the deamination of AMP into IMP by the action of adenylate deaminase (EC 3.5.4.6) (7, 8). Ammonia readily passes through the cell membrane, but most of the IMP may be preserved in the muscle and reconverted to AMP via adenylsuccinate (purine nucleotide cycle) (8). On the other hand, some of the IMP molecule may be broken down into its nucleoside, inosine, and its base, hypoxanthine (9, 10). These substances, which serve as precursors of uric acid, also can be detected in circulating blood, since the muscle cell membrane is relatively permeable to them. Although the physiological significance of intramuscular purine catabolism has not been thoroughly clarified, many data suggest that it is closely related to energy metabolism in exercising muscle (8, 10–16).

The present study was thus designed to investigate purine catabolism in the exercising muscles of patients with GSD types V and VII.

Methods

Patients. Two patients with GSD type V (patients 1 and 2) and three with type VII (patients 3–5) were studied. Patient 1, a 26-yr-old woman, was presented to our clinic because of her high plasma creatine kinase level at medical examination. The clinical details have been described elsewhere (17). Patient 2, a 29-yr-old woman, was referred to our clinic from Senboku National Hospital, Osaka. These two patients belonged to unrelated families. Patient 3, a 44-yr-old man, and patient 4, a 43-yr-old man, are brothers with GSD type VII from the family described previously (2). Patient 5, a 20-yr-old man with GSD type VII, belonging to the second family in Japan, was referred to our laboratory from Yokosuka Mutual Aid Hospital, Yokosuka, for biochemical analyses. Careful tracing of their histories revealed several episodes of a second wind phenomenon (18) in both cases of GSD type V but no evidence of the phenomenon in any of the GSD type VII cases. Definite diagnoses were established through enzymatic analyses of muscle biopsy specimens, and these data have been described elsewhere (2, 17, 19). A control group of unconditioned, healthy volunteers consisted of five men (19–38 yr old) and one woman (28 yr old). Informed consent was obtained from each of the patients and control subjects before the study.

Procedure. After bed rest for at least 30 min, ischemic forearm exercise tests were carried out, according to a procedure described previously (19). To determine lactate, inosine, and hypoxanthine levels, blood was deproteinized by adding 2 vol of cold 6% (vol/vol) perchloric
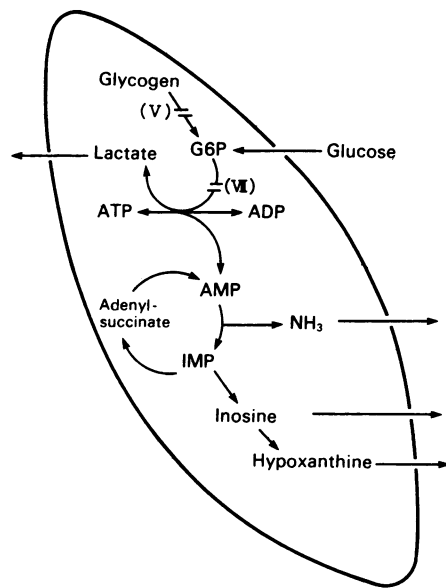
Figure 1. Reactions for purine catabolism and glycolysis in muscle. G6P, glucose-6-phosphate; (V) and (VII), metabolic block in GSD type V and type VII, respectively.

acid immediately after sampling. The extract was stored at −80°C until assayed. For plasma ammonia estimation, blood samples were collected in iced tubes containing crystallized heparin, and the assay was done within a few hours.

Assay. Blood lactate (20), plasma ammonia (21), and serum uric acid (22) were assayed enzymatically. Inosine and hypoxanthine were separated and quantitated by high performance liquid chromatography. The high performance liquid chromatography system (model 302, 802, 811, and 111B; Gilson Medical Electronics, Inc., Middleton, WI) with a microsorb C18 reversed-phase column (Rainin Instrument Co. Inc., Woburn, MA) was used. Chromatographic conditions were established according to the method of Zakaria et al. (23), the low-strength eluant consisting of a 20-mmol/liter potassium phosphate buffer (pH 5.7). A 60% (vol/vol) methanol-water mixture was used as the high strength eluant. The gradient was linear from 0 to 40% of the high strength eluant in 35 min. The elution peaks were identified by comparing retention times with known external standards and relative absorbances at 254:280 nm. Inosine and hypoxanthine for standard solution were purchased from Sigma Chemical Co., St. Louis, MO. Other chemicals were obtained from Wako Pure Chemical Industries, Ltd., Higashi-ku, Osaka, Japan.

Statistics. Differences in mean values between the two groups were studied by the unpaired t test. A possible correlation between a pair of parameters was sought by linear regression analysis.

Results

The concentrations of venous lactate, ammonia, inosine, hypoxanthine, and uric acid in the basal state (at 0 min) are given in Table I. Basal lactate lowered in every patient with GSD type V or VII. The mean value in the GSD group was significantly smaller than that in controls (544±37 vs. 882±54 μmol/liter, respectively, $P < 0.005$). Two patients with GSD type VII (patients 3 and 4) had very high concentrations of ammonia (118 and 79 μmol/liter, respectively), hypoxanthine (23.4 and 20.4 μmol/liter, respectively), and uric acid (14.3 and 11.9 mg/dl, respectively). Other patients with GSD type V (patients 1 and 2) and type VII (patient 5) showed near normal measurements.

The 2-min handgrip exercise caused no appreciable increase of venous lactate in any patient with GSD type V or VII (Fig. 2), indicating that intramuscular glycolysis never proceeded in vivo.

Venous ammonia levels increased after forearm exercise. In the control group, maximal increases of ammonia had a significant positive correlation with increases of lactate ($r = 0.841$, $P < 0.05$; data not shown). An exaggerated increase of ammonia was observed in every patient studied (Fig. 3), in contrast with the lack of lactate increase. The mean incremental area under the concentration curves of venous ammonia, calculated from the data in Fig. 3, was 13-fold greater in the GSD group than in the control group (1,120±182 vs. 83±26 μmol · min/liter) (Table II).

Venous inosine levels increased very little in the controls after forearm exercise, whereas the increase was prominent in patients with both types of GSD (Fig. 4). In patients with GSD type VII, inosine increased exponentially, with the concentrations at 8 min being >50 times higher than basal concentrations. The incremental area of inosine in each of the GSD patients was significantly greater than in any individual control subject, and the mean value in the GSD group was 73 times that in the control group (29.2±7.2 vs. 0.4±0.1 μmol · min/liter) (Table II).

Venous hypoxanthine levels were also much increased after forearm exercise in both types of GSD (Fig. 5). The mean incremental area of hypoxanthine in the GSD group was about nine times the control value (134.6±23.1 vs. 14.9±3.2 μmol · min/liter) (Table II). The incremental areas of ammonia in the control subjects and in the patients were plotted against those of hypoxanthine (Fig. 6), revealing a strong correlation between the two parameters ($r = 0.984$, $n = 11$, $P < 0.005$). This suggests that hypoxanthine was produced in proportion to the rate of AMP deamination (Fig. 1).

Table I. Basal Concentrations of Venous Lactate, Ammonia, Inosine, Hypoxanthine, and Uric Acid in Muscle GSD

	Lactate	Ammonia	Inosine	Hypoxanthine	Uric Acid
Patient 1 (type V)	572	35	0.6	2.3	3.4
Patient 2 (type V)	502	27	0.5	1.2	4.4
Patient 3 (type VII)	479	118	0.6	23.4	14.3
Patient 4 (type VII)	489	79	0.3	20.4	11.9
Patient 5 (type VII)	676	34	0.4	2.6	7.4
Controls (n = 6), mean±SE	882±54	23±4	0.7±0.1	2.0±0.4	5.3±0.6

Each value is represented in micromoles per liter plasma (ammonia) or blood (lactate, hypoxanthine, inosine) or milligrams per deciliter serum (uric acid).
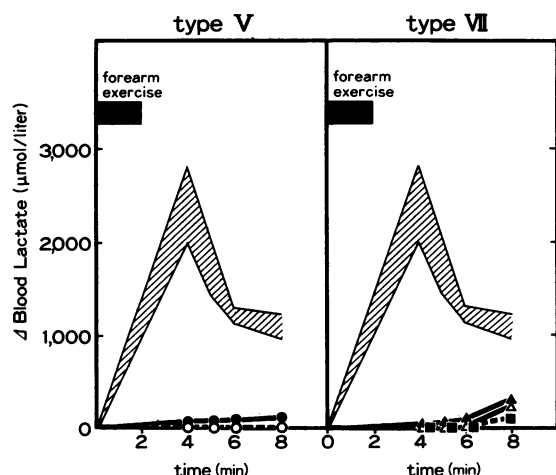
Figure 2. Increase in venous lactate concentration after ischemic forearm exercise in patients with GSD type V (patient 1, ●; patient 2, ○) and type VII (patient 3, ▲; patient 4, △; patient 5, ■). Forearm exercise was done by squeezing a hand dynamometer once every second for 2 min. Blood was drawn from an antecubital vein five times just before the start of exercise (0 min) and 4, 5, 6, and 8 min after exercise started. The shaded area indicates the mean±SE in healthy controls (*n* = 6).

Venous uric acid levels were not significantly changed after forearm exercise in either group (data not shown).

Discussion

Ammonia arises in exercising muscle from AMP deamination into IMP, the first step of the purine nucleotide cycle (Fig. 1). This study showed the exaggerated increase in venous ammonia levels in patients with types V and VII GSD after exercise (Fig. 3), thus providing the first evidence of an excess production of ammonia in GSD type VII. A similar increase in ammonia levels after exercise has already been shown in a patient with GSD type V (24). Moreover, a study on phosphorylase kinase-
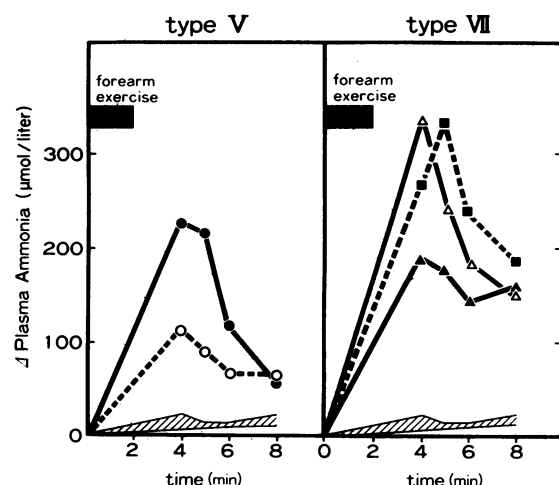
Table II. Incremental Areas of Venous Ammonia, Inosine, and Hypoxanthine after Forearm Exercise in Patients with Muscle GSD

	Σ Ammonia	Σ Inosine	Σ Hypoxanthine
Patient 1 (type V)	1,021	32.4	153.2
Patient 2 (type V)	535	5.8	51.2
Patient 3 (type VII)	1,032	45.5	121.2
Patient 4 (type VII)	1,463	41.4	166.3
Patient 5 (type VII)	1,551	21.1	181.1
Mean±SE	1,120±182*	29.2±7.2‡	134.6±23.1*
Controls (*n* = 6), mean±SE	83±26	0.4±0.1	14.9±3.2

The incremental areas (Σ) were estimated as the areas under the curves of venous ammonia, inosine, and hypoxanthine in Figs. 3, 4, and 5, respectively. Data represent micromoles times minutes per liter. The symbols (*, ‡) indicate significant differences ([*]$P < 0.001$, [‡]$P < 0.005$) from control values.

deficient mice revealed enhanced accumulation of IMP in exercising muscle (25). These findings suggest that purine catabolism is altered in the exercising muscles of patients with muscle-energy diseases affecting glycogenolysis and glycolysis.

Ammonia production by muscle is known to be greatest during intense exercise (26) or tetanic muscle stimulation (27), when the rate of ATP utilization may exceed the rate of ATP resynthesis. Meyer et al. (28) and Sabina et al. (15) recently demonstrated that the amount of IMP produced in exercising muscle is stoichiometric with the decrease in ATP. According to these observations, the exaggerated AMP deamination in patients with muscle GSD implies a critical shortage of ATP in their exercising muscles. Since the ATP pool in muscle is large and exercise performance in GSD is very limited, exercise-induced ATP depletion can hardly be detected (4, 5). Adenylate deaminase, an allosteric enzyme whose enzyme activity is regulated by many factors (e.g., pH, purine nucleotides, alkali metal ions, inorganic phosphate, enzyme binding to myosin, etc.; 8, 29, 30), is inhibited by ATP (at a low concentration),
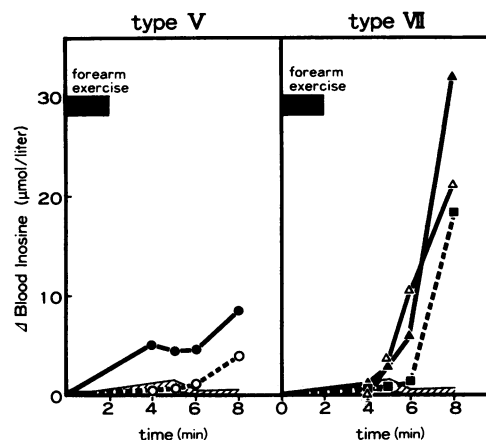

Figure 3. Increase in venous ammonia concentration after ischemic forearm exercise in patients with GSD type V (patient 1, ●; patient 2, ○) and type VII (patient 3, ▲; patient 4, △; patient 5, ■). The shaded area indicates the mean ±SE in healthy controls (*n* = 6).

Figure 4. Increase in venous inosine concentration after ischemic forearm exercise in patients with GSD type V (patient 1, ●; patient 2, ○) and type VII (patient 3, ▲; patient 4, △; patient 5, ■). The shaded area indicates the mean ±SE in healthy controls (*n* = 6).
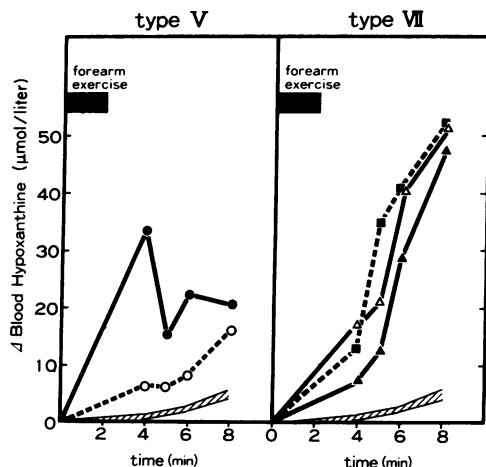
Figure 5. Increase in venous hypoxanthine concentration after isch-
emic forearm exercise in patients with GSD type V (patient 1, ●;
patient 2, ○) and type VII (patient 3, ▲; patient 4, △; patient 5, ■).
The shaded area indicates the mean ±SE in healthy controls (n = 6).

but activated by ADP and AMP. In muscle GSD, net ATP
hydrolysis must lead to a heavy accumulation of ADP and
AMP, due to the defect of ATP generation in glycolysis (Fig.
1). This derangement of adenine nucleotides may possibly be
responsible for the exaggerated AMP deamination.

In every patient with GSD type V or VII, forearm exercise
led to an exaggerated increase in venous inosine (Fig. 4) and
hypoxanthine (Fig. 5) as well as ammonia levels. Excess
hypoxanthine release from exercising muscle has been reported
in a patient with GSD type V (31). In the present study, we
showed a strong correlation between ammonia and hypoxan-
thine responses to forearm exercise (Fig. 6), suggesting that
increased AMP deamination may accelerate the subsequent
reactions of IMP breakdown to inosine, thereby leading to the
formation of hypoxanthine (Fig. 1). Thus, the more IMP
produced, the more inosine and hypoxanthine formed, both
of which can permeate the muscle cell membrane and appear
in circulating blood.

According to recent reports, some patients with GSD type
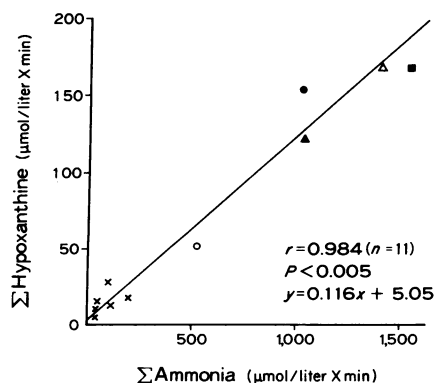VII (32–34) and muscle phosphoglycerate mutase deficiency

Figure 6. Relation of the incremental area of venous ammonia (Σ
ammonia) to that of hypoxanthine (Σ hypoxanthine) in the forearm
exercise tests. Data from Table II were used: control subjects (x),
patient 1 (●), patient 2 (○), patient 3 (▲), patient 4 (△), and patient 5
(■).

(35) develop hyperuricemia or gouty arthritis. Our patients
with GSD type VII (patients 3 and 4) also exhibited marked
hyperuricemia, and interestingly, the levels of venous ammonia
and hypoxanthine were conspicuously high even in the basal
state (Table I). We are now studying the etiological contribution
of excess purine degradation in exercising muscle to the
development of the hyperuricemia.

The basal concentration of venous lactate was significantly
lowered in every patient studied (Table I). We previously
demonstrated that glycolytic intermediates in resting muscles
from patients with GSD types V and VII showed a distinct
crossover at each of the phosphorylase and the phosphofruc-
tokinase steps (2, 17, 36), suggesting that the block in muscle
exists even at rest. The lowered basal lactate levels were
probably due to a defect of lactate production in muscle and
thus may be a sign suggestive of muscle-energy disease affecting
glycogenolysis or glycolysis. In addition, the exaggerated re-
sponses of ammonia, inosine, and hypoxanthine to exercise,
as well as the unresponsiveness of lactate (1, 2, 35, 37, 38),
may be useful indicators in screening patients for disturbed
intramuscular glycogenolysis and glycolysis.

In the present study, we demonstrated the excess release
of ammonia, inosine, and hypoxanthine (ATP catabolites)
from exercising muscles in patients with GSD types V and
VII. We considered a possible derangement of the ATP pool
due to defective intramuscular glycogenolysis or glycolysis.

Acknowledgments

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