Levels of erythrocyte glycolytic intermediates after the phosphofructokinase (PFK) step, including 2,3-bisphosphoglycerate (2,3-DPG), were decreased at rest in patients from separate families with type VII glycogenosis. The concentration of 2,3-DPG was about half of the normal control value during a period of unrestricted daily activity but was further decreased to one third of normal after a one-day bed rest. Mild ergometric exercise rapidly increased the levels of fructose-1,6-bisphosphate, dihydroxyacetone phosphate plus glyceraldehyde-3-phosphate, and 2,3-DPG in patients’ circulating erythrocytes but did not in those of normal subjects. This indicated that a crossover point at the PFK step in glycolysis disappeared after physical exercise and, consequently, the 2,3-DPG concentration, which had decreased because of blockage of the PFK step, was restored considerably. This apparently exercise-related alteration in intermediary metabolism at the beginning of glycolysis was reproduced in vitro by incubating normal erythrocytes in the presence of inosine or ammonia, both of which have increased levels in circulating blood during and after exercise in this disorder.

We conclude that physical activity in addition to a genetic deficiency in erythrocyte PFK affects glycolysis in erythrocytes in type VII glycogenosis and that myogenic factors released from exercising muscles may be responsible for this change.

## MATERIALS AND METHODS

### Patients

Erythrocytes from four patients with type VII glycogenosis were the subjects of this study. Patient 1 (a 46-year-old male) and patient 2 (a 43-year-old male) belong to the same family reported in 1965 as the probands of this disease. Patient 3 (a 19-year-old male) and patient 4 (a 16-year-old female) belong to another family in Japan. The kinetics of residual erythrocyte and liver PFK in all patients were similar. All the blood samples from the patients were drawn directly into a syringe containing 6% perchloric acid. Patient 2 exercised only intermittently on a bicycle ergometer because of exercise intolerance. The subject completed two 35-W work loads in five minutes with a ten-minute rest and then a 40-W load in eight minutes. Erythrocytes drawn from patient 2 after the bicycle ergometer exercise were also analyzed. Procedures and consent forms were approved by the Osaka University Hospital Committees on the Protection of Human Subjects in Research.

**Biochemical studies.** Glycolytic intermediates were assayed as described previously. The plasma ammonia concentration was measured enzymatically. Inosine was measured by a high-performance liquid chromatography system (Gilson) with a microorb C18 reversed-phase column (Rainin Instrument Co, Woburn, MA). The adenylate energy charge was calculated by the formula given by Atkinson and Walton.

An erythrocyte incubation study was performed as described previously. Leukocytes and platelets were removed from heparinized blood by filtration through a cellulose column. Then the erythrocytes were washed with 0.9% NaCl three times and suspended in a solution containing 100 mmol/L NaCl, 10 mmol/L KCl, 50 mmol/L triethanolamine/HCl, 30 mmol/L glucose, and 1 mmol/L inorganic phosphate (pH 7.4). The hematocrit of the suspension was 44%. After a ten-minute preincubation at 37°C, varying concentrations of ammonium chloride or inosine were added. The reaction was stopped by the addition of perchloric acid (final concentration, 3%) after 20 minutes.

All enzymes used in this study were purchased from Boehringer Mannheim and other reagents from Sigma Chemical Co (St Louis).

### RESULTS

**Erythrocyte metabolite concentrations at rest.** The concentration of glycolytic intermediates and adenine nucleotides in erythrocytes from patients with type VII glycogenosis at rest are shown in Table 1. There were decreases in fructose-1,6-bisphosphate, dihydroxyacetone phosphate plus glyceraldehyde-3-phosphate, and 2,3-DPG in all three patients examined at rest during the period of unrestricted...
daily activity. Pyruvate and lactate levels were also decreased. A consistent feature of type VII glycogenosis in all patients was a striking decrease in fructose-1,6-bisphosphate (43%) and 2,3-DPG (53%) levels. These changes were statistically significant (P < .001 and P < .001, respectively). The decreases in pyruvate (60%) and lactate (63%) levels were also statistically significant (P < .02 and P < .01, respectively).

There was a decrease in the concentration of total adenine nucleotides as well as adenosine triphosphate (ATP) and an increase in adenosine diphosphate (ADP) and adenosine monophosphate (AMP) in all patients. The changes in ATP and AMP were significant (P < .001 and P < .05, respectively). The adenylate energy charge value was significantly lower than in normal controls. The mean values of the erythrocyte metabolite concentrations in type VII glycogenosis are shown in Fig 1 in the form of a crossover plot. A crossover point was seen at the site between fructose-6-phosphate and fructose-1,6-bisphosphate, thus indicating metabolic blockage at the step of PFK.

The decrease in fructose-1,6-bisphosphate and 2,3-DPG levels was larger in two patients kept at bed rest for at least 24 hours, although the changes in the concentrations of other glycolytic intermediates and adenine nucleotides were similar to those observed before resting. The concentrations of fructose-1,6-bisphosphate and 2,3-DPG were 20% to 25% and 34% to 36% of controls, respectively. Sequential changes in 2,3-DPG concentrations during prolonged rest are shown in Fig 2. Levels of 2,3-DPG fell by about 800 μmol/L RBC during the first day and by 100 μmol/L RBC in the second day. Thus intensity of physical activity appears to further modify the intrinsically altered glycolytic intermediary metabolism in circulating erythrocytes in patients with type VII glycogenosis.

### Table 1. Erythrocyte Glycolytic Intermediates and Adenine Nucleotides in Type VII Glycogenosis

<table>
<thead>
<tr>
<th>Intermediate/Nucleotide</th>
<th>Normal Value (Mean ± SD)</th>
<th>During Unrestricted Daily Activity</th>
<th>During Bed Rest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient 1</td>
<td>Patient 2</td>
<td>Patient 3</td>
</tr>
<tr>
<td>G6P</td>
<td>44.5</td>
<td>38.4</td>
<td>46.4</td>
</tr>
<tr>
<td>F6P</td>
<td>13.9</td>
<td>12.2</td>
<td>14.4</td>
</tr>
<tr>
<td>FBP</td>
<td>0.92</td>
<td>0.70</td>
<td>0.64</td>
</tr>
<tr>
<td>DHP + GAP</td>
<td>14.7</td>
<td>8.2</td>
<td>7.2</td>
</tr>
<tr>
<td>2,3-DPG</td>
<td>2,950</td>
<td>2,430</td>
<td>2,320</td>
</tr>
<tr>
<td>3PGA</td>
<td>50.7</td>
<td>22.7</td>
<td>31.7</td>
</tr>
<tr>
<td>2PGA</td>
<td>5.8</td>
<td>7.1</td>
<td>4.7</td>
</tr>
<tr>
<td>PEP</td>
<td>14.5</td>
<td>15.5</td>
<td>9.9</td>
</tr>
<tr>
<td>PYR</td>
<td>56.1</td>
<td>61.6</td>
<td>58.7</td>
</tr>
<tr>
<td>LAC</td>
<td>605</td>
<td>597</td>
<td>513</td>
</tr>
<tr>
<td>ATP</td>
<td>1,404</td>
<td>1,170</td>
<td>1,140</td>
</tr>
<tr>
<td>ADP</td>
<td>243</td>
<td>254</td>
<td>304</td>
</tr>
<tr>
<td>AMP</td>
<td>36.8</td>
<td>31.7</td>
<td>47.7</td>
</tr>
<tr>
<td>Total adenylates</td>
<td>1,320</td>
<td>1,456</td>
<td>1,492</td>
</tr>
<tr>
<td>Energy charge</td>
<td>0.880</td>
<td>0.891</td>
<td>0.866</td>
</tr>
</tbody>
</table>

Values are expressed as μmol/L RBCs.

Abbreviations: G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; FBP, fructose-1,6-bisphosphate; DHP, dihydroxyacetone phosphate; GAP, glyceraldehyde-3-phosphate; 3PGA, 3-phosphoglycerate; 2PGA, 2-phosphoglycerate; PEP, phosphoenolpyruvate; PYR, pyruvate; LAC, lactate.

*Values in μmol/L blood.

**Effect of exercise on erythrocyte metabolism.** The effect of ergometric exercise on the glycolytic metabolism of erythrocytes in the systemic circulation was studied in a patient with type VII glycogenosis (patient 2). Immediately after exercise, concentrations of intermediates in the beginning of glycolysis were much increased, and those at the end became normal (Fig 3), whereas no significant change was found in normals (data not shown). The concentrations of...
fructose-1,6-bisphosphate and dihydroxyacetone phosphate plus glyceraldehyde-3-phosphate were about fourfold of control values. The AMP concentration became normal, and 2,3-DPG and ATP levels tended to increase during ergometric exercise. One hour after exercise, the concentrations of intermediates decreased but were still slightly higher than preexercise concentrations.

Although exercising was difficult for this patient, there was a significant increase in the 2,3-DPG concentration from 1,710 to 2,030 μmol/L RBC, but its concentration was not restored completely (Fig 2). To see the effect of prolonged but less intensive exercise on the 2,3-DPG concentration, 2,3-DPG was measured after the patient was discharged and had returned to work (the results are shown in Fig 2). Levels of 2,3-DPG increased from 2,010 to 2,420 μmol/L RBC, which is consistent with the finding that the 2,3-DPG concentration is decreased by prolonged rest.

In vitro studies. We previously showed that exercise on a bicycle ergometer markedly increased plasma inosine and hypoxanthine levels in this patient.12 The inosine concentration rose from 0.4 to 9.5 μmol/L and the ammonia concentration also rose from 60 to 475 μmol/L after exercise but was unchanged in normal subjects. To ascertain whether the exercise-related alteration of erythrocyte glycolysis in type VII glycogenosis is caused or mediated by the augmentation of inosine or ammonia, the effects of these metabolites on the intermediary metabolism of erythrocyte glycolysis were studied in vitro experiments, which are shown in Fig 4. The addition of inosine increased the levels of fructose-1,6-bisphosphate and dihydroxyacetone phosphate plus glyceraldehyde-3-phosphate. The increments of these intermediates were greater as the inosine concentration was increased up to 150 μmol/L. With the addition of 150 μmol/L inosine, 2,3-DPG levels increased from 4,280 to 4,730 μmol/L RBC in 20 minutes. The incremental amount of 2,3-DPG and lactate was more than the decremental amount of inosine, which may be in part due to the further activation of PFK by increased fructose-1,6-bisphosphate levels. The addition of ammonium chloride to give a final concentration of 1,000 μmol/L produced a forward crossover between fructose-6-phosphate and fructose-1,6-bisphosphate, thus indicating the increased flux at the PFK step.

DISCUSSION

Concentrations of erythrocyte glycolytic intermediates after the PFK step were decreased in two families with type VII glycogenesis (Table 1). This implies that the metabolic flux is decreased at the PFK step in vivo. The concentration of 2,3-DPG was about half that of normal during unrestricted daily activity and one third of that after bed rest. These changes in 2,3-DPG concentrations during different physical activities were observed in both families. Because erythrocyte PFKs of the patients from these families were

![Fig 2. Fluctuations of erythrocyte 2,3-DPG concentration by different physical activities in a patient with type VII glycogenosis. Patient 2 was kept in bed for two days after his admission. On the third day, exercise on a bicycle ergometer was performed before physical activities in a patient with different nc exercise. One hour plus glyceraldehyde-3-phosphate were about fourfold of control values. The AMP concentration became normal, and 2,3-DPG and ATP levels tended to increase during ergometric exercise. One hour after exercise, the concentrations of intermediates decreased but were still slightly higher than preexercise concentrations.](image1)

![Fig 3. Effect of ergometer exercise on the concentrations of glycolytic intermediates and adenine nucleotides in erythrocytes from patient 2 with type VII glycogenosis. The blood samples were obtained before (△), immediately after [▲], and one hour after [■] exercise. Concentrations are expressed in terms of the ratios to the normal resting values.](image2)

![Fig 4. Effect of ammonium and inosine on erythrocyte glycolytic intermediates. Erythrocytes were incubated at a Hct value of 44%. Incubation medium, 100 mmol/L NaCl, 10 mmol/L KCl, 50 mmol/L triethanolamine/HCl, 30 mmol/L glucose, and 1 mmol/L inorganic phosphate (pH 7.4). Concentrations are expressed in terms of the ratios to the controls. (A) △, 25 μmol/L; ▲, 50 μmol/L; ◇, 100 μmol/L; □, 150 μmol/L inosine. (B) ■, 250 μmol/L; ▲, 500 μmol/L; □, 1,000 μmol/L ammonium chloride.](image3)
similar both in velocity and in kinetics, the erythrocytes can be considered to share common metabolic defects. Figure 2 shows that physical activity clearly affected the level of 2,3-DPG in patient 2. During bed rest, 2,3-DPG levels gradually decreased and reached a nadir within one day; however, the increase in 2,3-DPG levels during exercise was very rapid, and the nadir values were similar to those in another family (Table 1).

Analysis of glycolytic intermediates in erythrocytes drawn immediately after exercise showed a marked increase in fructose-1,6-bisphosphate and dihydroxyacetone phosphate plus glyceraldehyde-3-phosphate levels (Fig 3). We also found that a similar increase occurred in types V and III glycogenoses in blood drawn from the antecubital vein after semiischemic forearm exercise (data not shown). Therefore, these alterations in levels of erythrocyte glycolytic intermediates after exercise seem to be common in muscle glycogenoses. These increases in levels of intermediates before 2,3-DPG in the glycolytic pathway may lead to a corresponding increase in the 2,3-DPG concentration. This effect of exercise lasted less than one hour. There were no changes in pH, O2 saturation, or serum inorganic phosphate levels in systemic blood during exercise, which are thought to affect the 2,3-DPG level. It has been reported that alanine is released from muscle but no uptake of branched-chain amino acids occurs in normal subjects during exercise, whereas in type V glycogenosis there is a marked uptake of alanine, leucine, and isoleucine by muscle without a significant release of other amino acids. In seeking other factors, we demonstrated previously that working muscle released ammonia and inosine to the local circulation in type V and VII glycogenoses and also found that even mild exercise could increase plasma ammonia and inosine levels in systemic blood in type VII glycogenosis. Ammonia is a potent activator of PFK, and inosine freely passes into erythrocytes and is metabolized rapidly through the hexose monophosphate shunt. Therefore, we performed an in vitro incubation study to determine the effect of these substances. The addition of ammonium chloride increased the levels of glycolytic intermediates after the PFK step only slightly, but the addition of ammonium chloride increased the levels of glyco-

Acknowledgment

We thank Dr W.G. Wilson for giving us helpful advice on English usage and M. Yoshihida for typing the manuscript.

References

Erythrocyte glycolysis and its marked alterations by muscular exercise in type VII glycogenosis

T Shimizu, N Kono, H Kiyokawa, Y Yamada, N Hara, I Mineo, M Kawachi, H Nakajima, YL Wang and S Tarui