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LOW GLUCOSE-1, 6-BISPHOSPHATE AND HIGH FRUCTOSE-2, 6-BISPHOSPHATE CONCENTRATIONS IN MUSCLES OF PATIENTS WITH GLYCOGENOSIS TYPES VII AND V

Yuya Yamada[1], Norio Kono[2], Hiromu Nakajima,
Takao Shimizu, Hiroaki Kiyokawa, Masanori Kawachi, Akira Ono,
Takamichi Nishimura, Masamichi Kuwajima, and Seiichiro Tarui

The Second Department of Internal Medicine, Osaka University Medical School,
1-1-50 Fukushima, Fukushima-ku, Osaka 553, Japan

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The level of glucose-1, 6-bisphosphate, a potent allosteric activator of phosphofructokinase, was markedly decreased in muscles of patients with glycogenosis type VII (muscle phosphofructokinase deficiency) and type V (muscle phosphorylase deficiency). Glucose-1-phosphate kinase activity in muscle was virtually absent in a patient with glycogenosis type VII, whereas it was normal in a patient with type V glycogenosis. Glucose-1-phosphate level was increased in type VII glycogenosis, whereas it was decreased in type V glycogenosis. Another activator of phosphofructokinase, fructose-2, 6-bisphosphate was increased in muscles of patients with both types of glycogenosis although it was much higher in type VII than in type V. This finding may be partly related to the difference of fructose-6-phosphate concentrations. The results suggest that phosphofructokinase would contribute to the major glucose-1-phosphate kinase activity in normal human muscle and would also form a kind of self-activating system.

Glucose-1, 6-bisphosphate (glucose-1, 6-P_2) and fructose-2, 6-bisphosphate (fructose-2, 6-P_2) are known to be potent allosteric activators of phosphofructokinase (EC 2.7.1.11), a key glycolytic enzyme (1, 2). In skeletal muscle, glucose-1, 6-P_2 plays an important role in the regulation of glycolysis (1), whereas fructose-2, 6-P_2 plays its main physiological role in the liver (2). In pig skeletal muscle, four enzymatic pathways participate in the biosynthesis of glucose-1, 6-P_2, and the reaction catalyzed by glucose-1-phosphate kinase (EC 2.7.1.10) (glucose-1-P kinase; glucose-1-P + ATP $\rightarrow$ glucose-1, 6-P_2 + ADP) has the highest activity (3). This reaction is also the main pathway of glucose-1, 6-P_2 synthesis in rabbit muscle (4). Since purified rabbit muscle phosphofructokinase has the activity of glucose-1-P kinase as a side reaction (4, 5), the examination of glycolytic metabolites in patients with glycogenosis type VII (muscle phosphofructokinase deficiency) may hold a key to the elucidation of glucose-1, 6-P_2 metabolism in human muscle. On the other hand, the biosynthesis of fructose-2, 6-P_2 is catalyzed by fructose-6-P 2-kinase. In patients with glycogenosis type V (muscle phosphorylase deficiency), as well as in those with glycogenosis type VII, the concentrations in muscles of glucose-1-phosphate (glucose-1-P) and fructose-6-phosphate (fructose-6-P), the substrate for glucose-1, 6-P_2 and fructose-2, 6-P_2 synthesis, respectively, may be altered as

[1] Present address; Internal Medicine, Sumitomo Hospital, 2-2 5-chome, Nakanoshima, Kita-ku, Osaka 530, Japan.
[2] To whom any correspondence should be addressed.

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a consequence of metabolic blocks. In this study, we measured the activity of glucose-1-P kinase and the concentrations of glucose-1, 6-P_2, fructose-2, 6-P_2 and glycolytic intermediates in muscle tissue from patients with glycogenosis types VII and V.

EXPERIMENTAL PROCEDURES

Three patients with glycogenosis type VII, six with glycogenosis type V and six control subjects were studied. Definite diagnoses were established through enzymatic analyses of muscle biopsy specimen. Patient 1 is a member of a family with glycogenosis type VII which was the first to be described (6). The nature of the enzyme defect of this family has been recently clarified at the DNA level (7). Patients 2 and 3 are siblings, and members of the second family in Japan as described elsewhere (8, 9). Patients 4 to 9 (type V glycogenosis) are unrelated. Clinical details regarding patients 4, 5 and 7 have been described elsewhere (8 - 12). Informed consent was obtained before the study. The research was carried out in conformity with *the Declaration of Helsinki.* Muscle tissue was obtained from the quadriceps femoral muscle of the patients with glycogenosis. Control muscle tissue was obtained during orthopedic surgery. All muscle tissue was immediately frozen between aluminum blocks in liquid nitrogen or between dry ice blocks and stored at -80°C until assays.

Muscle tissue was homogenized with 10 vol of 6% perchloric acid and centrifuged at 10,000g for 20 min at 4°C. The supernatant fraction was neutralized and glucose-1, 6-P_2 concentration was determined according to the procedure described by Eyer *et al.* (5). Fructose-2, 6-P_2 levels in muscle extracts, prepared as described (13) with minor modifications, were determined by the method of Van Schaftingen *et al.* (14). Glucose-1-P kinase activity was assayed according to the method described by Climent *et al.* (3) and Eyer and Pette (5). Concentrations of glycogen, glucose-1-P, glucose-6-phosphate (glucose-6-P), fructose-6-P, fructose-1, 6-bisphosphate (fructose-1, 6-P_2) and dihydroxyacetone-phosphate plus glyceraldehyde-3-phosphate were measured enzymatically (10, 15). Commercially available enzymes and substrates were purchased from Boehringer Mannheim and Sigma. Statistical analyses were made using the analysis of variance and Student's *t*-test.

RESULTS

Muscle glycogen content was increased from 2 to 5 fold in every patient with glycogenosis type VII or V (Table 1). In patients with glycogenosis type VII, hexose monophosphate pool, including glucose-1-P, glucose-6-P and fructose-6-P levels, was increased, whereas fructose-1, 6-P_2 and dihydroxyacetone-phosphate plus glyceraldehyde-3-phosphate levels were extremely low. The

Table 1
Concentrations of glycogen and glycolytic intermediates in skeletal muscle of patients with glycogenosis types VII and V

	Glycogen*	G-1-P	G-6-P	F-6-P	F-1, 6-P_2	Triose-P
Type VII						
Patient 1	38.2	66	1120	278	0.3	6.0
2	18.4	19	710	145	2.9	2.5
3	25.0	35	649	151	1.4	2.7
Type V						
Patient 4	39.4	4	50	11	5.0	9.0
5	27.2	7	41	6	20.0	15.0
6	51.8	4	62	17	8.8	7.9
7	23.2	4	26	13	5.1	3.1
8	36.1	ND	ND	ND	ND	ND
9	25.1	ND	ND	ND	ND	ND
Controls	11.4±4.2	19±5	415±164	79±26	66±50	53±31

Expressed as nmol/g muscle and *mg/g muscle. Control values are expressed as means±SD.
G-1-P, glucose-1-phosphate; G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; F-1, 6-P_2, fructose-1, 6-bisphosphate; Triose-P, dihydroxyacetone-phosphate + glyceraldehyde-3-phosphate; ND, not determined.

Table 2
Concentrations of glucose-1, 6-bisphosphate and fructose-2, 6-bisphosphate in muscle of patients with glycogenosis types VII and V

	G-1, 6-P_2	F-2, 6-P_2
Type VII		
Patient 1	2.3	25.1
2	2.1	6.5
3	3.2	14.4
Mean±SE	2.5±0.3***	15.3±5.4***
Type V		
Patient 4	2.1	2.6
5	1.1	2.4
8	6.6	1.6
9	0.7	1.9
Mean±SE	2.6±1.4***	2.1±0.2*
Controls	12.0±2.1	1.4±0.2

Expressed as nmol/g muscle. Control values are expressed as means±SD.
G-1,6-P_2, glucose-1, 6-bisphosphate; F-2,6-P_2, fructose-2,6-bisphosphate.
Statistical significance is indicated as * $p< 0.05$, ** $p< 0.02$, or
*** $p< 0.01$ versus control values.

metabolic block was observed to be at the step catalyzed by phosphofructokinase (fructose-6-P → fructose-1, 6-P_2). In patients with glycogenosis type V, levels of all intermediates in the first part of the glycolysis were markedly low, indicating the metabolic block to be at the step catalyzed by phosphorylase (glycogen → glucose-1-P). Mean glucose-1, 6-P_2 concentrations in patients with glycogenosis type VII and type V were only approximately 20 % of that in controls (Table 2). Glucose-1-P kinase activity in the muscle tissue of a patient with glycogenosis type VII (Patient 1) was less than 10% of the control value (0.82 U/g muscle versus 8.30±1.0 U/g muscle, mean±SE), whereas it was normal in muscle tissue from a patient with glycogenosis type V (Patient 9; 14.8 U/g muscle). Mean fructose-2, 6-P_2 concentration was increased more than 10 fold in glycogenosis type VII compared to that in controls (Table 2). In glycogenosis type V, fructose-2, 6-P_2 level was increased as well, but the increment was only small.

DISCUSSION

In this study, we demonstrated that glucose-1, 6-P_2 level was low and fructose-2, 6-P_2 level was high in muscle tissue from patients with glycogenosis types VII and V. In pig and rabbit skeletal muscle, the main route for the synthesis of glucose-1, 6-P_2 is reported to be glucose-1-P kinase reaction (3, 4). In pig skeletal muscle, two enzymes exhibit glucose-1-P kinase activity, one of which is associated with phosphofructokinase (3). Phosphofructokinase purified from rabbit skeletal muscle catalyzes phosphorylation of fructose-1-phosphate (fructose-1-P) and glucose-1-P as well as fructose-6-P (5, 16). The activity quotient of fructose-1-P kinase/fructose-6-P kinase is about 1/12 and that of glucose-1-P kinase/fructose-6-P kinase is about 1/150 (5, 16). However, whether human skeletal muscle phosphofructokinase has the activity of glucose-1-P kinase or not, has not been elucidated. In this study, we demonstrated that muscle glucose-1-P kinase activity was virtually absent (below 10 % of control values in Patient 1) and that muscle glucose-1, 6-P_2 levels were markedly low in patients with glycogenosis type VII. Thus we may conclude that glucose-1-P kinase deficiency is associated in a patient with muscle phosphofructokinase deficiency. The levels of muscle glucose-1, 6-P_2 were also low in patients with glycogenosis type V with normal ATP concentration (data not shown) and normal glucose-1-P kinase activity but low glucose-1-P

concentration. These data suggest that, in human skeletal muscle, glucose-1, 6-P_2 is mainly synthesized through glucose-1-P kinase, as it is in pig (3) and rabbit (4) muscle. The evidence was also shown that the major part of glucose-1-P kinase activity can be attributed to the side reaction of phosphofructokinase in human skeletal muscle. Since glucose-1, 6-P_2 is considered to be an important allosteric activator of phosphofructokinase in muscle (4, 5), these results also suggest that phosphofructokinase forms a kind of self-activating system in muscle.

Fructose-2, 6-P_2 concentration was high in muscle tissue from patients with glycogenosis types VII and V. In type VII, fructose-2, 6-P_2 concentration was about 10 fold higher than that in controls, and was comparable to that in rat liver (2, 13). The physiological significance of fructose-2, 6-P_2 in muscle has not been well characterized. The mechanism for this increase, common to both diseases, is unknown. Biosynthesis of fructose-2, 6-P_2 is catalyzed by fructose-6-P 2-kinase (EC 2.7.1.105) (fructose-6-P + ATP $\rightarrow$ fructose-2, 6-P_2 + ADP) and degradation by fructose-2, 6-bisphosphatase (EC 3.1.3.46) (fructose-2, 6-P_2 $\rightarrow$ fructose-6-P + phosphate) (2). The concentration of phosphoenolpyruvate, an inhibitor of fructose-6-P 2-kinase and an activator of fructose-2, 6-bisphosphatase, may be low due to the upstream metabolic block in both types of glycogenosis, which may in turn activate fructose-6-P 2-kinase or inhibit fructose-2, 6-bisphosphatase. The level of fructose-6-P, the substrate for fructose-6-P 2-kinase, was increased in type VII and, conversely decreased in type V. This may partly account for the large difference in fructose-2, 6-P_2 concentrations between two types of glycogenosis.

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REFERENCES

1. Beitner, R. (1985) *Regulation of carbohydrate metabolism*, pp 1 - 27, CRC Press, Inc., Florida.
2. Van Schaftingen, E. (1987) *Adv. Enzymol.* **59**, 315 - 395.
3. Climent, F., Carreras, M., and Carreras, J. (1985) *Comp. Biochem. Physiol.* **81B**, 737 - 742.
4. Eyer, P., Hofner, H.W., Krystek, E., and Pette, D. (1971) *Eur. J. Biochem.* **20**, 153 - 159.
5. Eyer, P. and Pette, D. (1967) *Life Sci.* **6**, 191 - 196.
6. Tarui, S., Okuno, G., Ikura, Y., Tanaka, T. Suda, M., and Nishikawa, M. (1965) *Biochem. Biophys. Res. Commun.* **19**, 517 - 523.
7. Nakajima, H., Kono, N., Yamasaki, T., Hotta, K., Kawachi, M., Kuwajima, M., Noguchi, T., Tanaka, T., and Tarui, S. (1990) *J. Biol. Chem.* **265**, 9392 - 9395.
8. Mineo, I., Kono, N., Shimizu, T., Sumi, S., Nonaka, K., and Tarui, S. (1984) *Muscle Nerve* **7**, 552 - 559.
9. Mineo, I., Kono, N., Shimizu, T., Hara, N., Yamada, Y., Sumi, S., Nonaka, K., and Tarui, S. (1985) *J. Clin. Invest.* **76**, 556 - 560.
10. Kono, N., Mineo, I., Sumi, S., Shimizu, T., Kang, J., Nonaka, K., and Tarui, S. (1984) *Neurology* **34**, 1471 - 1476.
11. Tabata, T., Kikunami, K., Matsushita, Y., Inoue, T., Inoue, T., Okamoto, T., Kono, N., Takahashi, M,. Tarui, S., and Morii, H. (1986) *Nephron* **44**, 371 - 374.
12. Mineo, I., Kono, N,. Hara, N., Shimizu, T., Yamada, Y., Kawachi, M., Kiyokawa, H., Wang, Y. L., and Tarui, S. (1987) *N. Engl. J. Med.* **317**, 75 - 80.
13. Yamada, Y., Shimizu, T., Hara, N., Mineo, I., Kawachi, M., Kiyokawa, H., Yamada, K., Fujioka, S., Matsuzawa, Y., Kono, N., and Tarui, S. (1988) *J. Biochem. (Tokyo)* **104**, 576 - 579.
14. Van Schaftingen, E., Lederer, B., Bartrons, R., and Hers, H.-G. (1982) *Eur. J. Biochem.* **129**, 191 - 195.
15. Kono, N., Kuwajima, M., and Tarui, S. (1981) *Diabetes* **30**, 346 - 353.
16. Uyeda, K. (1972) *J. Biol. Chem.* **247**, 1692 - 1698.