PHOSPHOFRUCTOKINASE DEFICIENCY IN SKELETAL MUSCLE. A NEW TYPE OF GLYCOGENOSIS

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This communication reports the biochemical elucidation of a specific enzyme defect in patients with a hitherto undescribed muscle disorder. Our observations were mainly based on three patients from a single family, and it was demonstrated that in all three there was an almost complete lack of muscle phosphofructokinase. Glycogenosis due to deficiency of muscle phosphorylase is known as McArdle's disease (McArdle, 1951; Mommaerts et al., 1959; Schmid and Mahler, 1959). In microorganisms evidence has been reported for the presence of a mutant lacking phosphoglucoisomerase (Fraenkel et al., 1963). However, until now there has been no report on the defect of a single enzyme in the glycolytic chain after the stage of G-1-P[1] in human muscles.

Three siblings, a 20 year old female (case 1) and 23 and 27 year old male adults (case 2 and case 3), all complaining of easy fatiguability and inability to keep pace with other persons, were recently referred to our laboratory for study. At rest no abnormalities were found by neurological examination. However, marked weakness and stiffness invariably appeared in muscle groups subjected to vigorous or prolonged exertion. In the ischemic exercise test, venous lactate failed to rise in all cases, and case 2 had a episode of

[1]The following abbreviations are used in the tables or text: glucose-1-phosphate, G-1-P; glucose-6-phosphate, G-6-P; fructose-6-phosphate, F-6-P; fructose-1,6-diphosphate, F-1,6-diP; glucose-1,6-diphosphate, G-1,6-diP.

brownish discoloration of the urine following the test. These cases had had the same symptoms since childhood. Their parents were first cousins. Both parents and two other siblings aged 17 and 25 could tolerate muscular exercise.

The three patients and both parents were available for biochemical analyses. The nature of the enzymes of their skeletal muscles and erythrocytes are described in the present paper.

Materials and Methods

Muscle specimens were obtained by biopsy of the quadriceps femoris under local anesthesia. Seven control biopsies of the quadriceps and gluteus maximus were obtained during surgical procedures. The glycogen was isolated by the method of Somogyi (1934) and determined using anthrone reagent, as described by Hassid and Abraham (1957). To test for alterations in the structure of the glycogen, the degradation rate of isolated glycogen was determined on exhaustive β-amylolysis (Sidbury *et al.*, 1961). Perchloric acid extracts of the muscles were assayed enzymatically for glycolytic intermediates, using coupled systems linked to reduction of TPN or DPN.

For studies *in vitro*, the muscle specimens were at once put into ice cold 1.5 per cent KCl solution and tested immediately. Anaerobic glycolysis was studied by a slight modification of the method of Schmid and Mahler (1959). Lactate was estimated in duplicate by the method of Barker and Summerson (1941). Phosphorylase activity was determined by the method described previously (Okuno *et al.*, 1964). For assays of the activities of other glycolytic enzymes, muscle specimens were homogenized in an all glass homogenizer with 20 volumes of cold Tris-EDTA buffer (0.05 M Tris, 5 mM EDTA, pH 7.5) and then centrifuged for 30 minutes at 80,000 x *g*; assays were carried out at 24°C in a 1 ml cuvette in coupled systems in which the enzymes to be measured were rate limiting; phosphoglucomutase and phosphoglucoisomerase activities were measured in a system coupled with G-6-P dehydrogenase; phosphofruktokinase was coupled with aldolase, triose-P isomerase and glycerophosphate dehydrogenase;

pyruvate kinase was coupled with lactic dehydrogenase. The protein in the sample fluids was measured by the biuret method (Gornall et al., 1949).

Fresh blood was obtained by venipuncture using heparin as an anticoagulant; hemolysates were prepared by freezing and thawing an aqueous suspension of erythrocytes, previously washed twice with isotonic saline solution. Phosphofructokinase in the hemolysates was assayed by the same method employed for muscle extracts.

Results and Discussion

Table 1. Glycogen and Glycolytic Intermediates in Muscle Specimens

	Glycogen (% degradation by β-amylase)	G-6-P*	F-6-P*	F-1,6-diP*
	mg/100mg wet weight		μmoles/g wet weight	
Case 1	4.38 (46.6%)	9.2	1.6	0.02
Case 2	1.56 (46.5%)	4.6	0.86	0.03
Case 3	2.90 (48.6%)	2.3	0.38	0.04
Father	0.92 (44.4%)	0.38	0.05	0.43
Mother	0.59	0.18	0.05	0.68
Normals (6) M.±s.d.	0.96 ±0.18 (44.3 ±5.7%)	0.50 ±0.30	0.10 ±0.05	0.61 ±0.23

* The tissue was homogenized in 10 per cent perchloric acid; then, a neutralizing mixture containing 1 M Tris buffer, pH 8.0, and 4 M KOH was added; after removal of $KClO_4$, the sample fluid was analyzed enzymatically for glycolytic intermediates; G-6-P was determined with G-6-P dehydrogenase and TPN essentially according to the method of Slein (1950); after the reaction of G-6-P with the dehydrogenase was completed, F-6-P was determined by adding phosphoglucoisomerase to the reaction mixture; F-1,6-diP was determined with glyceraldehyde-3-P dehydrogenase, aldolase and DPN by the method of Cori et al. (1948).

Table 1 shows the accumulation of glycogen and hexose monophosphates and the extreme fall in the diphosphate concentration in the muscles of the patients. Although the ratio of G-6-P to F-6-P remained constant in all the materials studied, the ratio of F-6-P to F-1,6-diP in the muscles of the patients was 60- to 500-fold higher than in normal muscles. Since the conversion of F-6-P to F-1,6-diP is considered not to be significantly reversible, these data are interpreted as reflecting a markedly lowered activity of phosphofructokinase. On β-amylolysis the degradation rates of

the glycogen extracted from the muscles of the patients were found to be within the normal range, indicating that the accumulated glycogen had a normal branched structure.

In the anaerobic glycolysis (Table 2) evidence was also obtained, showing the presence of a metabolic block between F-6-P and F-1,6-diP in the muscles of the patients. The lactate production in the materials from the patients was very low in the presence of hexose monophosphates, although F-1,6-diP was metabolized in the abnormal muscles (case 1 and case 2) more than twice as rapidly as in normal muscles.

Table 2. Anaerobic Glycolysis by Muscle Homogenates with and without Added Substrates

Substrate	Case 1	Case 2	Case 3	Normal Mean (4)
	μmoles lactate formed/30 mins./g wet weight			
No Addition	0(9.7)	0(0)	0(5.6)	0(21.1)
Glycogen	0	7.6	4.8	20.4
G-1-P	25.5	19.8	19.2	131
G-6-P	17.7	6.9	10.6	105
F-6-P	4.5	12.2	12.0	93
F-1,6-diP	384	311	193	149

The values in parentheses indicate lactate production without added substrate.
In experiments in which substrate was added, 20 μM of hexose monophosphate or diphosphate, or 5.4 mg of animal glycogen was present per flask.

Table 3 summarizes determinations of the activities of the individual enzymes in the glycolytic sequence. Very little activity of phosphofructokinase was specifically demonstrated in the muscles of the patients, being between approximately 1 and 3 per cent of the normal mean activity. The muscle specimen from the mother had a phosphofructokinase activity which was on the borderline of the normal range. No remarkable alterations were observed in the activities of phosphoglucomutase and phosphoglucoisomerase. It is of interest that pyruvate kinase activity was significantly elevated in the

Table 3. Activities of Glycolytic Enzymes in Muscle Specimens

	Phosphorylase*	PG-M**	PG-I**	PF-K**	PYR-K**
Case 1	25.8	0.82	0.81	0.002	4.5
Case 2	18.6	1.01	0.95	0.004	5.0
Case 3	21.6	0.91	0.54	0.005	7.5
Father	21.4	0.80	0.49	0.24	3.5
Mother	15.6	0.72	0.36	0.16	1.9
Normals (5-7) M.±s.d.	19.0 ±2.8	0.80 ±0.14	0.57 ±0.19	0.19 ±0.03	2.0 ±0.1

* mg inorg. P liberated/10 mins./g wet weight
** μmoles substrate converted/min./mg protein
PG-M : Phosphoglucomutase; PG-I : Phosphoglucoisomerase
PF-K : Phosphofructokinase; PYR-K : Pyruvate kinase
The assay mixture for PG-M contained 0.04 M Tris buffer, pH 7.5; 0.01 M thioethanol; 2 mM $MgCl_2$; 2 mM G-1-P; 2 μM G-1,6-diP; 0.5 mM TPN; and G-6-P dehydrogenase 0.28 unit. The assay mixture for PG-I contained 0.04 M Tris buffer, pH 7.5; 6 mM $MgCl_2$; 0.5 mM F-6-P; 0.5 mM TPN; and G-6-P dehydrogenase 0.28 unit. The assay mixture for PF-K contained 0.05 M Tris buffer, pH 8.0; 0.01 M thioethanol; 1 mM $MgCl_2$; 1 mM ATP; 1 mM F-6-P; 0.15 mM DPNH; aldolase 0.5 unit; triose-P isomerase 1.0 unit; and glycerophosphate dehydrogenase 0.33 unit. The assay mixture for PYR-K contained 0.05 M Tris buffer, pH 7.4; 0.1 M KCl; 5 mM $MgCl_2$; 2 mM ADP; 2 mM phosphoenolpyruvate; 0.15 mM DPNH; and lactic dehydrogenase 0.9 unit.

muscles of the patients. It is presumably involved in compensatory mechanisms, giving a possible explanation for the increased lactate production in the presence of F-1,6-diP during anaerobic glycolysis of muscle homogenates of the patients.

The muscle derives its energy almost exclusively from the breakdown of glycogen to lactate during vigorous muscular activity and phosphofructokinase plays a key role in the Embden Meyerhof glycolytic pathway. Therefore, it is reasonably to conclude that the intolerance of exercise in these patients is caused by a deficiency of phosphofructokinase. This disease is also regarded as a new type of glycogenosis characterized by the marked accumulation of hexose monophosphates and moderate deposition of glycogen.

An enzymatic study of the erythrocytes (Table 4) also demonstrated an obviously lowered activity of phosphofructokinase in the materials from the

patients. It is apparent, however, that the enzyme in the erythrocytes is only partially affected in contrast to the almost complete lack of phosphofructokinase in muscle. Careful analyses of the erythrocytes from the mother revealed slightly but definitely lowered phosphofructokinase activity.

Table 4. Phosphofructokinase Activity in the Hemolysates

	μmoles substrate converted/min./ml RBC
Case 1	0.41
Case 2	0.66
Case 3	0.58
Father	1.37
Mother	0.89
Normals (9) M.±s.d.	1.37 ± 0.40

The occurrence of this disease in siblings of both sexes, which were offspring of a consanguineous mating, indicates that it is a hereditary metabolic disorder. Assuming it to be a single recessive autosomal type of inheritance, both parents should be heterozygous carriers of the disease trait. Although the minor deviations from the normal average in the phosphofructokinase activities of materials from the mother is in favor of this assumption, data on materials from the father are not, and further work is needed on this problem.

Summary

A new type of glycogenosis due to deficiency of muscle phosphofructokinase is described. It occurred in three siblings from a single family, who had suffered from intolerance of exercise since childhood. This disease is characterized by the marked accumulation of hexose monophosphates and moderate glycogen deposition in skeletal muscles. Erythrocyte phosphofructokinase is only partially affected in this disease in contrast to the almost complete lack of muscle phosphofructokinase.

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