CHANGES IN GLUCOSE 1,6-DIPHOSPHATE AND IN THE ACTIVITIES OF PHOSPHOFRUCTOKINASE, PHOSPHOGLUCOMUTASE AND GLUCOSE 1,6-DIPHOSPHATE PHOSPHATASE INDUCED BY FASTING AND REFEEDING IN DYSTROPHIC MUSCLE

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1. Introduction

Recent studies have revealed that glucose 1,6-diphosphate (Glc-1,6-P_2), which was first shown by Leloir et al. [1–3] to be the coenzyme in the phosphoglucomutase reaction, acts as a potent regulator common to several key enzymes in carbohydrate metabolism. The levels of this regulator were found to be strikingly reduced in muscle from genetically dystrophic mice [4,5]. Since Glc-1,6-P_2 is one of the strongest activators (deinhibitors) of phosphofructokinase [6–8], the rate-limiting enzyme in glycolysis, the reduction in Glc-1,6-P_2 levels in the dystrophic muscle could offer an explanation for the observed reduction in the allosteric activity of phosphofructokinase and glycolysis in the dystrophic muscle [4]. Our results have also revealed that the decrease in Glc-1,6-P_2 in dystrophic muscle may be attributed to the increase in the activity of Glc-1,6-P_2 phosphatase (the enzyme which degradates Glc-1,6-P_2) [9]. Based on our experiments which have shown that the Glc-1,6-P_2 levels in muscle change under various physiological and hormonal conditions [10–13], we investigated the effect of fasting and refeeding on the levels of this regulator in muscle of normal and dystrophic mice, and examined whether the changes in Glc-1,6-P_2 induced by these conditions are associated with concomitant changes in the activities of phosphofructokinase and phosphoglucomutase. We also investigated the changes in Glc-1,6-P_2 phosphatase activity under these conditions.

The results here show that the decrease in Glc-1,6-P_2 and the concomitant reduction in the activities of phosphofructokinase and phosphoglucomutase which occur in muscular dystrophy, can be reversed by the fasting-refeeding treatment, which reduces Glc-1,6-P_2 phosphatase activity to normal level.

2. Methods

Normal and genetically dystrophic mice of strain 129 ReJ, aged 4–7 weeks, were used. The animals were divided into three groups: fed ad libitum, fasted for 22 h, or fasted for 22 h and then refed for 9 h. Mice were anesthetized with 60 mg/kg sodium pentobarbital, hind-leg muscles were removed and rapidly frozen between a pair of aluminium tongs precooled in liquid N_2. Frozen muscles were powdered in a mortar cooled in liquid N_2, and the powder was used for extraction of Glc-1,6-P_2 and enzymes.

Glc-1,6-P_2 was extracted and measured as in [5]. Phosphofructokinase was extracted and assayed spectrophotometrically at pH 6.9 (regulatory conditions) and at pH 8.2 (maximal, non-regulatory conditions) as in [4,13]. 1 munit of phosphofructokinase activity catalyzed the formation of 1 nmol fru-1,6-P_2 per min at 25°C. Phosphoglucomutase and Glc-1,6-P_2 phosphatase were extracted in 10 volumes of 0.02 M Tris–HCl (pH 7.0) and the homogenate was centrifuged at 4°C for 10 min at 27 000 × *g*. Glc-1,6-P_2 phosphatase was assayed as in [9]. 1 munit of Glc-1,6-P_2 phosphatase activity catalyzed the degradation of 1 nmol of Glc-1,6-P_2 per 15 min at 37°C. Phosphoglucomutase was assayed spectrophotometrically measuring both regulatory and maximal

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activity as in [13]. 1 munit of phosphoglucomutase activity catalyzed the reduction of 1 nmol of $NADP^+$/min at 25°C. Protein was measured by the method in [14]. Non-collagen protein was determined by the method in [15].

3. Results

As shown in fig.1, the levels of Glc-1,6-P_2 in muscle of normal mice were markedly reduced after 22 h of fasting. When the fasted normal mice were refed for 9 h, the Glc-1,6-P_2 returned to initial levels. In muscle of dystrophic mice, where the Glc-1,6-P_2 levels were much lower than normal, 22 h fasting induced a proportionally similar reduction in Glc-1,6-P_2. However, when the fasted dystrophic mice were refed, the Glc-1,6-P_2 levels were not only restored to the initial dystrophic level, but dramatically reached the levels of the normal muscle.

Since Glc-1,6-P_2 is a potent activator of phosphofructokinase and phosphoglucomutase, in the experiments demonstrated in tables 1 and 2, we examined whether a correlation exists between the striking changes in Glc-1,6-P_2 during fasting and refeeding and the activities of these enzymes. As can be seen from table 1, phosphofructokinase activity, when measured under regulatory conditions (i.e., when the enzyme is subject to regulation by Glc-1,6-P_2 and its other allosteric effectors [13,16–19]), was in full correlation to Glc-1,6-P_2 levels (fig.1). It was reduced by fasting and increased by refeeding. (The changes in the enzyme's activity under all conditions, were similar, whether the results were expressed per mg protein or per mg non-collagen protein.) In the dystrophic muscle, where the enzyme activity was significantly lower than normal, there was a proportionally similar response to fasting, but a strikingly greater sensitivity to refeeding; phosphofructokinase activity was almost completely restored to normal values, in muscle of the refed dystrophic mice. Phosphofructokinase activity was also measured at pH 8.2, under conditions known to be optimal for its activity, where the enzyme shows no regulatory (allosteric) properties [13,16–19]. Under these maximal conditions, there was no significant difference in phosphofructokinase activity of the normal or dystrophic

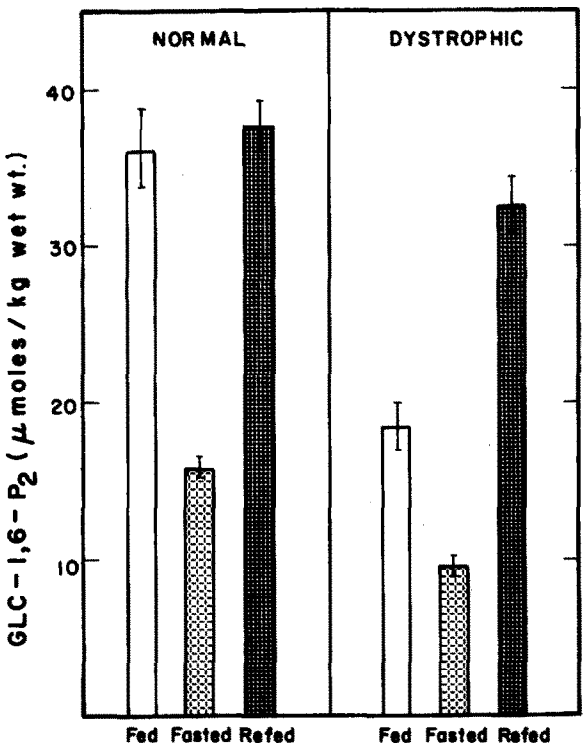

Fig.1. The effect of fasting and refeeding on Glc-1,6-P_2 levels in muscle of normal and dystrophic mice. Values are means ± SEM for 5–10 animals in each group. *P* values (fasted vs. fed; refed vs. fasted; dystrophic fed vs. normal fed) <0.005.

mice, whether fed, fasted or refed. Similar results were obtained for phosphoglucomutase activity (table 2). The regulatory activity, measured under submaximal conditions, where the enzyme is sensitive to activation by Glc-1,6-P_2 [13] correlated with the intracellular changes of Glc-1,6-P_2 (fig.1); it was significantly lower in the dystrophic muscle, and was reduced by fasting and increased by refeeding both in the normal and dystrophic muscle. Similar to phosphofructokinase, the maximal activity of this enzyme (measured under optimal conditions, and in the presence of saturating concentration of Glc-1,6-P_2 [13]) did not change through all the different experimental states.

Trying to understand the interesting changes in Glc-1,6-P_2 induced by fasting and refeeding of both normal and dystrophic mice (fig.1), we measured the activity of Glc-1,6-P_2 phosphatase (the enzyme that

Table 1
The effect of fasting and refeeding on phosphofructokinase activity in normal and dystrophic muscle

	Phosphofructokinase activity			
	Regulatory activity		Maximal activity	
	mU/mg protein	mU/mg NCP[a]	mU/mg protein	mU/mg NCP[a]
Normal				
Fed	230 ± 12	283 ± 20	802 ± 23	987 ± 29
Fasted	118 ± 10	153 ± 11	749 ± 14	918 ± 12
Refed	287 ± 24	369 ± 33	740 ± 54	987 ± 69
Dystrophic				
Fed	63 ± 5	81 ± 6	706 ± 15	918 ± 84
Fasted	27 ± 3	35 ± 3	641 ± 95	812 ± 96
Refed	162 ± 11	223 ± 21	677 ± 36	923 ± 37

[a] NCP, non-collagen protein

Values are means ± SEM for 6–10 animals in each group

P values: regulatory activity (fasted vs. fed; refed vs. fasted; dystrophic fed vs. normal fed) <0.005; maximal activity, not significant

degradates Glc-1,6-P_2). Table 3 shows marked increase in Glc-1,6-P_2 phosphatase activity in the dystrophic muscle. Fasting induced an elevation in the enzyme's activity in both normal and dystrophic mice and refeeding reduced its activity. In the refed dystrophic mice there was a most dramatic decrease in Glc-1,6-P_2 phosphatase activity, falling to the levels of a normal fed mouse.

Table 2
The effect of fasting and refeeding on the activity of phosphoglucomutase in normal and dystrophic muscle

	Phosphoglucomutase activity			
	Regulatory activity		Maximal activity	
	mU/mg protein	mU/mg NCP[a]	mU/mg protein	mU/mg NCP[a]
Normal				
Fed	256 ± 13	315 ± 20	2350 ± 81	2830 ± 130
Fasted	87 ± 9	129 ± 16	2350 ± 100	2870 ± 100
Refed	280 ± 14	371 ± 17	2300 ± 70	2870 ± 150
Dystrophic				
Fed	132 ± 20	169 ± 30	2266 ± 348	2750 ± 443
Fasted	88 ± 11	111 ± 16	2178 ± 228	2650 ± 320
Refed	177 ± 20	227 ± 27	2157 ± 90	2600 ± 112

[a] NCP, non-collagen protein

Values are mean ± SEM for 6–10 animals in each group

P values: regulatory activity (fasted vs. fed; refed vs. fasted; dystrophic fed vs. normal fed) <0.002; maximal activity, not significant

Table 3
The influence of fasting and refeeding on the activity of Glc-1,6-P_2 phosphatase in normal and dystrophic muscle

	Glc-1,6-P_2 phosphatase activity	
	mU/mg protein	mU/mg NCP[a]
Normal		
Fed	0.30 ± 0.01	0.36 ± 0.02
Fasted	0.45 ± 0.04	0.53 ± 0.05
Refed	0.21 ± 0.01	0.28 ± 0.01
Dystrophic		
Fed	0.66 ± 0.01	0.84 ± 0.05
Fasted	0.89 ± 0.01	1.02 ± 0.04
Refed	0.29 ± 0.05	0.37 ± 0.05

[a] NCP, non-collagen protein

Values are means ± SEM for 6–10 animals in each group

P values (fasted vs. fed; refed vs. fasted; dystrophic fed vs. normal fed) <0.005

4. Discussion

The results presented in this paper reveal a striking correlation between the tissue levels of Glc-1,6-P_2 and the activities of phosphofructokinase and phosphoglucomutase which are activated by this regulator. Fasting induced a reduction in Glc-1,6-P_2 and a concomitant reduction in the activities of both enzymes in normal and dystrophic muscle, whereas refeeding induced a rise in Glc-1,6-P_2 accompanied by an enhancement in these enzymes' activities. The close correlation between intracellular levels of Glc-1,6-P_2 and the enzymes' activities was also found in our earlier studies under various other physiological and hormonal conditions [10,12,13]. The results strongly suggest that the changes in phosphofructokinase and phosphoglucomutase activities induced by fasting and refeeding are mediated by changes in the intracellular concentration of the regulator, and are not due to changes in the total content of these enzymes in the cell. This suggestion is based on the fact that the changes in enzymes' activities were demonstrated only when assayed under regulatory (submaximal) conditions (which most probably represent the native state of the enzymes in muscle cell [18,20,21], whereas their maximal activities, namely, their full catalytic potential, remained unchanged (tables 1, 2).

It is interesting that the dystrophic muscle is much more sensitive to refeeding than the normal muscle. Refeeding of the fasted dystrophic mice results in an overshooting of Glc-1,6-P_2 and a concomitant rise in the activities of phosphofructokinase and phosphoglucomutase, reaching values of the normal control mice. These results suggest that refeeding may reverse the reduction in glycolytic capacity of the dystrophic muscle, by raising the concentration of Glc-1,6-P_2. Since the activity of Glc-1,6-P_2 phosphatase, which rises markedly in the dystrophic muscle, is dramatically reduced to normal levels in the refed dystrophic mice (table 3), it offers an explanation to the mechanism by which Glc-1,6-P_2 rises so strikingly following refeeding (fig.1). The factor responsible for inhibition of this enzyme's activity during refeeding is at present under investigation in our laboratory.

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