Oxygen Consumption and Anaerobic Glycolysis of the Muscle from the Patients with Progressive Muscular Dystrophy

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In a previous paper of ours, the authors reported that the decrease of creatinephosphokinase was particularly remarkable as compared with that of the other enzymes in the muscle of progressive muscular dystrophy (PMD) and that a disturbance in energy storage system due to the decrease of creatinephosphokinase might be the first lesion in metabolism of the affected muscle in this particular disorder.

We extended our investigation in order to observe the overall efficiency of the energy yielding system in the affected muscle by determining the oxygen consumption and anaerobic glycolysis in it.

METHODS AND MATERIALS

Muscle specimens, weighing about 2 g, were removed surgically from the gastrocnemius of five patients with PMD (all patients were of the Duchenne type except Case 1 which was of the limb-girdle type). As controls, orthopedic patients without muscular disorder were subjected to muscle biopsy. Immediately after the removal of muscle specimens, the tissue slices were prepared at 0°C. A part of the specimens was subjected to histological examination.

Manometric estimation of oxygen consumption and of anaerobic glycolysis in the muscle slices was made as follows: For the estimation of oxygen consumption, the incubation flasks of the Warburg manometer, containing 2.5 ml of Krebs-Ringer-phosphate buffer and muscle slice in the main chamber, 0.5 ml of 20% KOH in the central well and 10 mg of sodium succinate (0.2 ml) in the side-arm, were shaken at 37°C for two hours under the atmosphere of air, and the oxygen consumption during the incubation was manometrically estimated. For the estimation of anaerobic glycolysis, the incubation flasks containing 2.5 ml
of Krebs-Ringer-bicarbonate solution and muscle slice in the main chamber and 10 mg of glucose (0.2 ml) in the side-arm, were shaken at 37°C for two hours under the atmosphere of 95% N₂ and 5% CO₂, and the content of lactic acid produced during the incubation was manometrically estimated. The estimation was started when glucose or sodium succinate was transferred from the side-arm into the medium following ten minutes' shaking for temperature equilibrium.

RESULTS AND DISCUSSION

Histological findings of the biopsied muscle specimens are shown in Fig. 1. Case 1 (the limb-girdle type): Muscular destruction is mild. Most of muscle fibers remain intact. The beginning picture of muscular destruction is in part seen.

Fig. 1. Histological findings of the muscle from the patients with progressive muscular dystrophy (Hematoxylin-eosin stain, 100 ×).
Muscular Dystrophy: Oxygen Consumption & Anaerobic Glycolysis

Case 2 (the Duchenne type): Muscular destruction is severe. Variation in size of muscle fibers, hyaline degeneration and fatty infiltration are remarkable.

Case 3 (the Duchenne type): Muscular destruction is moderate. Partially, hyaline degeneration and fatty infiltration are seen.

Case 4 (the Duchenne type): Muscular destruction is severe. Variation in size of muscle fibers, hyaline degeneration and fatty infiltration are remarkable.

Case 5 (the Duchenne type): Muscular destruction is moderate. Hyaline degeneration and fatty infiltration are partially seen.

Table I showed the results of manometric estimation of oxygen consumption and anaerobic glycolysis in the muscle of the patients. The oxygen consumption (respiration) of the affected muscle was diminished in value per 1 g of the muscle, but was of almost the same level in value per 1 mg of nitrogen in the muscle, namely on protein basis, as compared with that of the controls (cf. Fig. 2). This suggests that the intact part of the muscle may have maintained normal activity of TCA cycle. The anaerobic glycolysis of the affected muscle was definitely diminished in value per 1 g of the muscle and was considerably diminished even on protein basis as compared with that of the controls (cf. Fig. 3).

Table I. Oxygen Consumption and Anaerobic Glycolysis by the Muscle of Progressive Muscular Dystrophy

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>Oxygen consumption QO2 µl O2/h</th>
<th>Anaerobic glycolysis QCO2 N2 µl CO2/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control average</td>
<td>4 (5.32 (26.3))</td>
<td>6 (3.02 (15.3))</td>
</tr>
<tr>
<td>Range</td>
<td>4.43 - 6.30 (23.7 - 29.3)</td>
<td>2.70 - 3.30 (13.5 - 17.1)</td>
</tr>
<tr>
<td>Case 1</td>
<td>3.20 (58.0)</td>
<td>1.62 (12.1)</td>
</tr>
<tr>
<td>Case 2</td>
<td>4.45 (26.5)</td>
<td>0.45 (5.7)</td>
</tr>
<tr>
<td>Case 3</td>
<td>2.05 (23.3)</td>
<td>1.57 (9.3)</td>
</tr>
<tr>
<td>Case 4</td>
<td>3.30 (29.5)</td>
<td>0.71 (8.1)</td>
</tr>
<tr>
<td>Case 5</td>
<td>3.02 (15.3)</td>
<td>1.22 (10.7)</td>
</tr>
</tbody>
</table>

Results are given as values per 10 mg of the muscle.
Figures in parenthesis indicate value per 1 mg of nitrogen in the muscle.

It seemed likely that the more advanced the stage of muscle degeneration was, the more remarkably decreased the anaerobic glycolysis; the decrease of anaerobic glycolysis on protein basis was milder in the muscles of Cases 1 and 5 which exhibited a relatively early stage of muscular destruction (cf. Fig. 1) than in the muscles of Cases 2 and 4 which exhibited an advanced stage of muscular destruction (cf. Fig. 1).
Why should such a discrepancy between the activity of respiration and that of anaerobic glycolysis in the affected muscle take place? This might be due to the enzymes involved in glycolysis, which are present in the soluble fraction in the cytoplasm, are easy to leak from the muscle as compared with the enzymes catalyzing the TCA cycle, which are bound to mitochondria. This view may be supported by the evidence that glycolytic enzymes such as aldolase, phosphorylase, or phosphoglucomutase are diminished in the muscle of the patients with PMD while the respiratory enzymes are not.

Ronzoni et al. have suggested that the decrease of the enzymes in the muscle of this particular disorder might be due to an alteration in permeability of the muscle cell membrane permitting a loss of the enzymes from the muscle since the activities of these enzymes are found to be increased in serum of the patient. Such a decrease of enzymes in the muscle of PMD appeared to differ in severity according to the kinds of enzyme. According to Ronzoni et al., creatinephosphokinase, aldolase and phosphorylase are greatly deficient, but hexokinase and lactic dehydrogenase are not deficient in the muscle of PMD. As was described in the previous paper, we found that the decrease of creatinephosphokinase was more remarkable than that of aldolase or lactic dehydrogenase in the muscles from the patients in an early stage of PMD. It is, therefore, supposed that a disturbance in energy storage system due to the decrease of creatinephospho-
kinase may be the first phase of metabolic derangement in the muscle of PMD. There is an evidence that creatinephosphate is on protein basis noticeably decreased but that ATP is on protein basis within normal limits in the muscle of PMD. \[1,4,6\] This evidence will support that the disturbance in energy storage system consisting of creatinephosphate may precede the disturbance in energy yielding system.

At present the mechanism of energy transfer in muscular contraction is generally interpreted as follows (cf. Fig. 4). Nervous impulse acts on myosin (ATPase), a component of muscular fiber, which decomposes ATP to ADP. The energy produced by breakdown of ATP is directly utilized for muscular contraction. At the same time, the decreased ATP is regenerated by creatinephosphokinase: Creatinephosphate + ADP $\rightarrow$ ATP + creatine. When supply of oxygen becomes insufficient during the muscular contraction, the anaerobic glycolysis of glycogen to lactic acid takes place. The energy produced through anaerobic glycolysis is transferred to ATP and, by creatinephosphokinase, to creatinephos-
phate. Thus ATP and creatinephosphate are constantly supplemented, so that they are not wanting throughout the period of muscular contraction. At recovery period from muscular contraction, lactic acid yielded during muscular contraction returns to pyruvic acid which is oxidized to CO₂ and H₂O by TCA cycle. The energy produced by oxidation through TCA cycle is transferred to creatinephosphate by way of ATP. At the same time the regeneration of glycogen from blood sugar takes place.

Now we would like to presume, basing upon our own results, to discuss the derangement in mechanism of muscular contraction in PMD. The energy production (regeneration of ATP by TCA cycle) at recovery period may be intact because the affected muscle showed almost normal capacity of oxygen consumption on protein basis. This will explain that ATP is not diminished on protein basis in the affected muscle.¹ ⁴ ⁶ However, the storage of energy in creatinephosphate may be insufficient because of a decrease of creatinephosphokinase. This will probably explain that the muscle of PMD is deficient in creatinephosphate.¹ ⁴ ⁶ In the contraction period of muscle, the supplement of ATP from creatinephosphate may be disturbed because of the decrease of creatinephosphokinase. Although ATP should be in part supplied through anaerobic glycolysis during the contraction period, our own results demonstrated that anaerobic glycolysis by the affected muscle would be decreasing with the progress of the disorder. Such a disturbance in anaerobic glycolysis may serve to aggravate the deficiency of ATP during the muscular contraction, resulting in the decrease of muscular power.

Fig. 4. Schema of energy transfer in muscular contraction.
SUMMARY

The oxygen consumption and anaerobic glycolysis by the muscle from the patients with progressive muscular dystrophy were manometrically investigated.

The oxygen consumption, on protein basis, of the affected muscle was within normal limits. Its anaerobic glycolysis was, on protein basis, considerably diminished. The decrease of anaerobic glycolysis seemed to be proportionate to the severity of muscular destruction.

In addition to the disturbance in energy storage system due to the decrease of creatinephosphokinase, the decrease of anaerobic glycolysis also may play a part in causing the decrease of muscular power in this particular disorder.

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Reference