Muscle Energetics during Exercise by $^{31}$P NMR

Shin-ya Kuno and Yuji Itai

Department of Radiology, Institute of Clinical Medicine,
University of Tsukuba

Previous studies have shown that a decrease in muscle tension is not proportional to decrease in the ATP concentration and free energy for ATP degradation but is proportional to the decrease in ATP consumption during muscle contraction, and that the free ADP concentration in the cell can be estimated using the Pi/PCr ratio obtained by $^{31}$P NMR. From this view, we discuss findings on muscle energetics during exercise that have been clarified by $^{31}$P NMR and its future problems.


Key words: $^{31}$P NMR, Muscle energetics, Exercise

1. Need of NMR studies

Even a single cell in living organisms consists of several compartments divided by membranes such as the cytoplasm, nucleus, and mitochondria. Its structure is closely associated with biological functions. Higher organisms show a systematic stratification such as tissues and organs and are involved in higher life activities as a whole. Progress in biochemistry and molecular biology due to advances in methods of separation and analysis has provided much information on individual enzyme reactions and the mechanism of genes. However, to clarify the kinetics and regulation of enzyme reactions in the cell and higher biological functions regulated by hormones and nerves, methods, unlike conventional ones, that analyze the ecosystem at the original state without destroying it are needed. Among various physiochemical analytic methods, NMR spectroscopy may be the most appropriate for this purpose due to the following characteristics. 1) The magnetic field used in NMR has high permeability for living organisms. 2) NMR requires very slight energy for measurement and is non-invasive. 3) Its resolution is high, and information at the molecular level can be obtained even in complicated heterogeneous mixture systems such as living organisms.

2. Muscle fatigue and NMR

Wilkie and Dawson at London University and Gadian et al. at Oxford University (1977, 1980) applied $^{31}$P NMR examination while electrically stimulating the muscle of living frogs. Twenty minutes after the initiation of stimulation, a decrease in phosphocreatine (PCr) and an increase inorganic phosphate (Pi) were observed, but ATP did not decrease. The rate of the decrease in PCr was nearly equal to the rate of the increase in Pi. After 75 minutes, each reaction was further enhanced, and ATP slightly decreased (Fig. 1). On the other hand, the decrease in muscle tension was not proportional to the decrease in the ATP concentration or free energy for ATP degradation that was calculated from the ADP concentration obtained from the equilibrium constant for creatine kinase. The decrease in muscle tension was proportional to the decrease in ATP consumption during muscle contraction. These findings suggested that the decrease in muscle tension during contraction is caused not by decreases in the ATP concentration...
and free energy for ATP degradation but by a decrease in ATP consumption. Gadian et al. (1981) examined whether the creatine kinase reaction (PCr + ADP ⇌ Cr + ATP) is in equilibrium in the cell not only at rest but also during muscle contraction by the magnetization transfer method using $^{31}$P NMR. This had not been clarified because the measurement of free ADP in the cell was difficult. They measured reaction fluxes in the positive and reverse directions by the magnetization transfer method. The two fluxes were nearly similar at rest, and the reaction was in equilibrium (1.6 μmol·g⁻¹·s⁻¹). During muscle contraction, the flux in the positive direction for ATP production was similar to that at rest while the flux in the reverse direction decreased to a value about half the flux in the positive direction. Since the flux for PCr degradation during muscle contraction was 0.75 μmol·g⁻¹·s⁻¹, the flux for the creatine kinase reaction in the positive reaction was larger than this. This explains the negligible changes in the ATP concentration during contraction.

In these studies, though the physiologically state was maintained by perfusion, the method was invasive. Subsequently, improvement in apparatuses has allowed measurement in humans and animals. Data obtained by non-invasive methods are introduced in the following.

3. Non-invasive studies in the human skeletal muscle

Taylor et al. (1983) calculated the PCr and Pi concentrations in the muscle of the human forearm at rest from $^{31}$P NMR spectra. The PCr concentration was 24–25 mM. Considering that the extracellular PCr concentration is about 1 mM, information obtained by $^{31}$P NMR primarily represents intracellular data. Intracellular pH can be also measured by $^{31}$P NMR (Dawson et al. 1977; Fig. 2). This is calculated from different chemical shifts that correspond to pH, utilizing that the peak of Pi is markedly affected by pH. A decrease in intracellular pH is considered to be nearly proportional to the accumulation of lactic acid in the muscle. Therefore, the measurement of pH is also useful for evaluating accumulated lactic acid. Lactic acid can be directly measured by $^1$H NMR, but its changes during exer-
cise are difficult to evaluate due to methodological problems of NMR.

Fig. 3 shows the relationship between the PCr/ (PCr+Pi) ratio and intracellular pH at rest and during exercise. When the ratio decreased below 40 %, pH rapidly decreased. Since the decrease in intracellular pH is correlated with lactic acid production, the flexion point observed here seems to indicate the conversion point for activation of the glycolysis system. This activation of the glycolysis system may have been induced by increases in intracellular ADP and AMP concentrations at this point were 42 and 0.3 μM, respectively. This diverging point at a PCr/(PCr+Pi) ratio of 40 % was confirmed by our NMR study during exercise using the thigh (Inaki et al. 1991). In addition, the MGH group of Harvard University recently performed 31P NMR examination during progressive exercise in combination with expired gas analysis and measurement of blood lactic acid and observed a marked decreased in pH at about 65 % \( \dot{VO}_{2\text{max}} \) (Systrom et al. 1990; Fig. 4). Since this decrease was in agreement with the ventilation threshold (VT) and lactic acid threshold (LT), especially closely with VT, they suggested that a decrease in pH accelerates ventilation.

In the above experiment by Taylor et al., ATP did not decrease during exercise. Subsequently, they determined 31P NMR spectra during exercise until exhaustion and reported a decrease in ATP during exercise in 6 of 12 subjects (Taylor et al. 1986). The PCr concentration and pH during the maximal exercise were lower in the group showing no changes in ATP. In addition, they calculated the concentration of each compound and free energy for ATP degradation from results of NMR examination (Table 1). The ADP concentration in the group showing a decrease in ATP was nearly twice the value in the other group, but free energy for ATP degradation was similar between the two groups. These findings suggest that muscle fatigue is independent of decrease in the ATP concentration, supporting the above results reported by Dawson et al. (1977, 1980).

Another interesting finding in this study was that the ATP concentration that decreased during exercise did not recover even 40 minutes after discontinuation of exercise. Such a decrease was also observed in an experiment using horses (Harris and Snow 1984). Compared with ATP, PCr and Pi recovered.

**Fig. 3** Relationship between intracellular pH and PCr/ (PCr+Pi) at rest and during exercise (Taylor et al. 1983)

**Fig. 4** Relationship between intracellular pH and % \( \dot{VO}_{2\text{max}} \) at incremental exercise (Systrom et al. 1990)
Table 1  The concentration of phosphorus metabolites during exercise (Taylor et al. 1986)

<table>
<thead>
<tr>
<th>Type of exercise</th>
<th>n</th>
<th>pH  (±0.03)</th>
<th>ATP (mM)</th>
<th>PCR (mM)</th>
<th>Pi (mM)</th>
<th>Total P (mM)</th>
<th>ADP (μM)</th>
<th>A (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (rest)</td>
<td>13</td>
<td>7.02</td>
<td>8.2</td>
<td>38.2±2.1</td>
<td>4.1±0.7</td>
<td>69±5</td>
<td>6±3</td>
<td>61±2</td>
</tr>
<tr>
<td>Light exercise</td>
<td>10</td>
<td>6.87±0.09</td>
<td>8.5±0.8</td>
<td>20.0±6.9</td>
<td>22±6</td>
<td>70±7</td>
<td>45±22</td>
<td>52±1</td>
</tr>
<tr>
<td>5 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exhaustion no ATP</td>
<td>6</td>
<td>6.37±0.09</td>
<td>7.7±0.6</td>
<td>10.0±4.0</td>
<td>35±9</td>
<td>73±12</td>
<td>43±20</td>
<td>52±1</td>
</tr>
<tr>
<td>depletion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exhaustion ATP</td>
<td>6</td>
<td>6.12±0.04</td>
<td>4.4±1.0</td>
<td>6.4±2.4</td>
<td>42±8</td>
<td>69±10</td>
<td>22±9</td>
<td>51±2</td>
</tr>
<tr>
<td>depletion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A: free energy for ATP degradation

Fig. 5  Recovery of PCR, Pi and pH from exhaustion exercise (Taylor et al. 1986)

early. However, the recovery was delayed in the group showing a decrease in ATP compared with the other group (Fig. 5). PCR recovered rapidly in the early stage and gradually thereafter. In this early stage, the rate of recovery in the group showing a decrease in ATP was 3 times slower than in the other group. On the other hand, pH recovered slowly in the early stage at similar rates between the two groups. However, it recovered rapidly thereafter, and the rate of recovery was slower in the group showing a decrease in ATP.

These results suggest that recovery of PCR is useful for evaluating oxidation by exercise. In particular, recovery of PCR in the early stage may be due to ATP re-synthesis of high energy phosphate is important. In this recovery process, PCR is involved in the increase in energy reserve. However, ADP is re-synthesized by phosphorylation of ADP and not by conversion of PCR. The slow recovery of PCR in the group showing a decrease in ATP may have been associated with a higher H⁺ concentration and the decreased ATP concentration.

4. Evaluation of training effects by ³¹P NMR

The group led by Dr. Chance in Pennsylvania University (1981, 1985, 1986) determined NMR spectra while quantifying exercise by connecting a cybex machine, which allows measurement of isokinetic muscle strength, to NMR. McCully et al. (1989) compared the muscle in the forearm between first-rate oarsmen and sedentary controls. Evaluation of the relationship between the Pi/PCr ratio and produced muscle strength during progressive exercise of endurance showed a smaller Pi/PCr in the oarsmen at similar muscle strength (Fig. 6). In
addition, when the Pi/PCr ratio at the final time of exercise was made the same between the two groups, its subsequent recovery was more rapid in the oarsmen. They calculated the velocity (V_{max}) of an oxidative ATP formation rate, regarding the produced muscle strength as metabolic activity and the Pi/PCr ratio as the substrate concentration and assuming that the intracellular concentration regulates respiratory function (Chance et al. 1981, 1986), and the Pi/PCr ratio is proportional to free ADP (Chance et al. 1986, Taylor et al. 1986). The V_max of ATP synthesis was higher in the oarsmen than the controls. Subsequently, Kuno et al. (1991) subjected rats to 6-month endurance training and evaluated the muscle in the hindlimb by 31P NMR and confirmed about 40% increase in the V_max of an oxidative ATP formation rate compared with the control group.

McCully et al. (1989) attributed higher oxidation in the oarsmen to higher 1) muscle fiber recruitment, 2) muscle blood flow, and 3) muscle oxidative enzyme capacity. They showed an increase in blood flow and oxidative enzyme activity by endurance training but did not clarify whether the training effects are also observed during sub-maximal exercise. Since inadequate oxygen supply is not considered to be a limiting factor during sub-maximal exercise (Chance et al. 1986), their hypothesis has not been yet confirmed. They also measured H_2PO_4^- and HPO_4^{2-} that have recently attracted attention (Wilkie 1986), and evaluated their relationship with muscle strength during lump loading. Muscle strength was not proportional to H_2PO_4^- of course, the H_2PO_4^- concentration was lower in the oarsmen than in the controls. Therefore, limiting factor in muscle fatigue may include not only an increase in H_2PO_4^- concentration but also other factors.

Fig. 7 shows the changes in intracellular pH during one season in Japanese athletes (Kuno unpublished data). NMR examination was serially performed after two sets of 3-minute exercise with 30-sec rest between them in May (off season), July (summer competition period), September (immediately before the Japan League), and December (during league). The amount of exercise was similar at the 4 times of measurement. However, the decrease in intracellular pH during exercise was the smallest in December during the league. These results suggest that muscle energy metabolism is markedly affected by the state of training even in top athletes. Such serial measurements during one season are possible in athletes because allows non-invasive evaluation of muscle energetics.
REFERENCES


(Received March 5, 1992)