Thresholds for Decrease in Intracellular pH and Increase in Blood Lactate during Progressive Exercise: $^{31}$P-MRS Study

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The purpose of this study was to compare the intramuscular and the intravascular events in relation to energy metabolism during progressive arm exercise. Twelve healthy untrained Japanese males participated in this study as subjects. They performed wrist flexion in a ramp incremental load of 0.14 W/min until exhaustion. $^{31}$P-MR spectra were obtained from wrist flexor muscle before and throughout the exercise. Venous blood was also sampled from antecubital vein with one minute interval during the exercise, and a change in plasma lactate concentration (La) was observed. Intracellular pH (pH) was calculated from a chemical shift between phosphocreatine (PCr) and inorganic phosphate (Pi) of the $^{31}$P-MR spectra. Change in pH showed a threshold behavior during exercise. Threshold points at decline in pH (pHT), increase in Pi/PCr (PT), and increase in La (LT) were determined by piecewise linear regression analysis of minutes-by-minutes data. Mean values of pHT, PT and LT were 43.0, 42.5, and 24.8% of maximal work rate, respectively. LT was significantly smaller than pHT and PT. This result suggests that lactic acidosis has already existed when pH is kept at resting level, and pHT reflects the capacity of remaining intracellular biochemical homeostasis, which might be one of the physiological indices of muscle fatigue.


Key words: Intracellular pH, Blood lactate, $^{31}$P-MRS

Since Wasserman and McIlroy had reported the concept of anaerobic threshold (AT) in 1964 (Wasserman and McIlroy, 1964), AT has been a topic of great interest not only in the field of respirocirculatory physiology during exercise but also in clinical medicine and sports sciences. AT was defined as "the level of work or O$_2$ consumption just below that at which metabolic acidosis and the associated changes in gas exchange occur (Wasserman et al., 1973)". On the other hand, it has been reported that there is a threshold on lactate accumulation in the working muscle (Chwalbinska-Moneta et al., 1989; Ivy et al., 1987; Jorfeldt et al., 1978; Kunttgen and Saltin, 1972). Chwalbinska-Moneta et al. (1989) demonstrated the similar lactate threshold (LT) for muscle and blood by using muscle biopsy technique. However, the difficulty of continuous measurement of the technique prevents an accurate detection of intracellular "threshold point". Development of in vivo magnetic resonance spectroscopy (MRS) allows us to measure intracellular events during exercise, continuously and non-invasively. Using $^{31}$P-MRS, Systrøm et al. (1990) reported the earlier threshold of decline in intramuscular pH than that of blood lactate accumulation. We discussed, however, the possibility that acid-base equilib-
rium was well balanced by intracellular buffer system though lactic acidosis has already existed at a lower muscular contraction level, and then the pH threshold did not reflect the onset of lactate production by glycolysis (Iwanaga et al., 1992).

The aim of the present study is to verify the above hypothesis. Our strategies of this study are a use of ramp exercise protocol to detect the threshold point by analysis of minutes-by-minutes data, and simultaneous measurements of $^{31}$P-MRS and blood lactate concentration.

METHODS

Subjects. Twelve untrained Japanese males, ranging in age from 26 to 34 yr, participated as subjects. Before an experiment, each subject was informed of the risks, benefits, and procedure of the study, and gave their written informed consent.

Exercise protocol. The subjects performed wrist flexion by holding a handle and depressing it when their right arm was attached in the bore of MR magnet. Exercise load was increased by injection of water at a constant rate of 250 ml/min into a waterproof bag lifted on the end of the crank arm connected to the handle. Injection rate of water was kept constant by the physical principle of the difference in vertical position between water sink and outlet of tube into the bag. Ramp slope in the work rate calculated from the water injection rate and the lifting distance of the bag by wrist flexion was 0.14 watt (W)/min. The subject performed the exercise initiated 0.025 W including the weight of empty bag and crank arm, and continued it until all out at a frequency of 0.5 Hz. All out point was defined as the work rate at which the subject could not lift the bag at that frequency.

Measurement. Prior to the experiment, nonmagnetic sensitive canula was inserted into the antecubital vein of the right arm. Venous blood was sampled before exercise and at one minute intervals during the exercise. Plasma lactate (La) concentration was analyzed by enzymatic method.

$^{31}$P-MRS was performed before exercise for 2 minutes and throughout the exercise. Each spectrum consisted of a sum of 60 and 30 scans for at rest or during exercise, respectively. From the spectra, intracellular pH was calculated from chemical shift ($\sigma$) between phosphocreatine (PCr) and inorganic phosphate (Pi) according to the following equation (Taylor et al., 1983):

$$pH = 6.75 + \log \left( (\sigma-3.27)/(5.69-\sigma) \right)$$

Pi-to-PCr ratio (Pi/PCr) was obtained from the peak heights of them on a spectrum.

The MR spectrometer used in this study had a superconducting magnet with operating frequency at 34.05 and 84.12 MHz for $^{31}$P and $^1$H, respectively. Double-tuned surface coil for $^{31}$P and $^1$H of 4 cm of diameter was used to apply the radio frequency pulses of $31 \mu$sec of pulse width at 2 second intervals, and detect the resonance phenomena. Surface coil was placed under the wrist flexors.

Determination of threshold points. Thresholds in pH (pHT), Pi/PCr (PT) and La (LT) were determined by means of piecewise regression analysis as an intersection of two linear regression lines. Log (Pi/PCr) plotted against work rate was analyzed to determine PT (Marsh et al., 1991) and log (La) vs log (work rate) to LT (Beaver et al., 1985; Systrom et al., 1990). Decision of two regression lines were based on the criterion of minimizing the residual sum of square (RSS) for estimation by the two regression equations (Vieth, 1989), or subjective observation if a join point by a pair of lines of minimum RSS did not coincide with changing point obviously.

Statistical analysis. Statistical difference between the means of threshold was observed by one-factor analysis of variance (ANOVA). Significance was set at $p<0.05$. Results of the groups are expressed as means and standard errors of mean (SE).

RESULTS

Figure 1 presents the changes in pH, Pi/PCr and La against work rate in one subject. Regression
Fig. 1 Changes in intracellular pH, Pi/PCr and plasma lactate (La) during wrist flexion in one of the subjects. pH, PT and LT show the thresholds for pH, Pi/PCr and La, respectively. Regression analysis of semilog and log-log transformations for detection of PT and LT are also presented.

Table 1  pH, PT and LT of each subject expressed in work rate (W).

<table>
<thead>
<tr>
<th>Subj.</th>
<th>pH</th>
<th>PT</th>
<th>LT</th>
</tr>
</thead>
<tbody>
<tr>
<td>IW</td>
<td>0.780</td>
<td>1.132</td>
<td>0.398</td>
</tr>
<tr>
<td>UE</td>
<td>0.741</td>
<td>---</td>
<td>0.394</td>
</tr>
<tr>
<td>UC</td>
<td>0.964</td>
<td>0.989</td>
<td>0.341</td>
</tr>
<tr>
<td>KH</td>
<td>0.695</td>
<td>0.991</td>
<td>0.862</td>
</tr>
<tr>
<td>KY</td>
<td>1.095</td>
<td>1.096</td>
<td>0.814</td>
</tr>
<tr>
<td>KM</td>
<td>1.787</td>
<td>1.749</td>
<td>0.412</td>
</tr>
<tr>
<td>SK</td>
<td>1.183</td>
<td>0.938</td>
<td>0.700</td>
</tr>
<tr>
<td>HD</td>
<td>0.399</td>
<td>0.337</td>
<td>0.710</td>
</tr>
<tr>
<td>MK</td>
<td>0.509</td>
<td>---</td>
<td>0.504</td>
</tr>
<tr>
<td>MT</td>
<td>1.196</td>
<td>1.137</td>
<td>0.241</td>
</tr>
<tr>
<td>MM</td>
<td>0.999</td>
<td>0.835</td>
<td>0.458</td>
</tr>
<tr>
<td>WT</td>
<td>2.583</td>
<td>1.282</td>
<td>1.297</td>
</tr>
</tbody>
</table>

Mean  1.078 a  1.049 b  0.594 a
SE  0.173  0.112  0.086

Symbols a and b show significant differences between mean values with a different symbol by one-factor ANOVA (p<0.05).

DISCUSSION

Threshold on decline in intramuscular pH during incremental muscle contraction had already been reported by Systrom et al. (1990) and Marsh et al. (1991). Also in the present study, a change in pH during wrist flexion showed a threshold behavior. Decrease in intramuscular pH has been discussed as one of the important causes of muscle fatigue (Sahlin, 1986; Edwards, 1986). Some studies showed that a decrease in intracellular pH inhibited the activity of phosphofructokinase which is a key enzyme in anaerobic energy metabolism (Ahlborg et al., 1972; Donaldson and Hermansen, 1978). Nakamura and Schwarts (1972) demonstrated that an increase in (Ca++) binding protein caused by a decrease in muscle pH inhibited muscle contraction. Thus, pH is supposed to be an index of muscle fatigability from the viewpoint of intracellular biochemical environment, because the homeostasis of acid-base
Fig. 2  Relationships between pHT, PT and LT expressed in work rate (W) and percent of maximal work rate (%). Dotted lines show identity lines (y = x). Significant correlation was found only in pHT vs PT in W.
Table 2  pH\(_T\), PT and LT of each subject expressed in percentage of maximal work rate (% WR\text{max}).

<table>
<thead>
<tr>
<th>Subj</th>
<th>pH(_T)</th>
<th>PT</th>
<th>LT</th>
</tr>
</thead>
<tbody>
<tr>
<td>IW</td>
<td>32.5</td>
<td>47.2</td>
<td>16.6</td>
</tr>
<tr>
<td>UE</td>
<td>30.6</td>
<td>----</td>
<td>16.3</td>
</tr>
<tr>
<td>UC</td>
<td>41.4</td>
<td>42.5</td>
<td>14.6</td>
</tr>
<tr>
<td>KH</td>
<td>32.3</td>
<td>46.1</td>
<td>40.1</td>
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<tr>
<td>KY</td>
<td>56.2</td>
<td>56.2</td>
<td>41.7</td>
</tr>
<tr>
<td>KM</td>
<td>54.0</td>
<td>52.8</td>
<td>12.5</td>
</tr>
<tr>
<td>SK</td>
<td>39.3</td>
<td>31.2</td>
<td>23.3</td>
</tr>
<tr>
<td>HD</td>
<td>18.4</td>
<td>15.5</td>
<td>32.7</td>
</tr>
<tr>
<td>MK</td>
<td>27.8</td>
<td>----</td>
<td>27.5</td>
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<tr>
<td>MT</td>
<td>58.3</td>
<td>55.5</td>
<td>11.8</td>
</tr>
<tr>
<td>MM</td>
<td>45.8</td>
<td>38.3</td>
<td>21.0</td>
</tr>
<tr>
<td>WT</td>
<td>79.5</td>
<td>39.4</td>
<td>39.9</td>
</tr>
<tr>
<td>Mean</td>
<td>43.0(^a)</td>
<td>42.5(^a)</td>
<td>24.8(^b)</td>
</tr>
<tr>
<td>SE</td>
<td>4.8</td>
<td>3.9</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Symbols of a and b show significant differences between mean values with a different symbol by one-factor ANOVA (p<0.01).

equilibrium is kept well before pH\(_T\).

It has been considered that intramuscular acidosis (acidemia) during exercise is explained by lactate production owing to anaerobic glycolysis (Sahlin, 1986). So, pH\(_T\) might be easily considered as coinciding with onset of La production or glycolysis. Systrom et al. (1990) reported in a \(^{31}\)P-MRS study that pH\(_T\) appeared at 66.4 % of VO\(_2\text{max}\) earlier than LT at 78.6 % of VO\(_2\text{max}\). They discussed pH\(_T\) reflected the threshold at the onset of glycolytic energy metabolism. In the present study, however, the contrary result was observed, i.e., pH\(_T\) at 43.0 % of WR\text{max} vs LT at 24.8 % of WR\text{max}. This result suggests that La production resulted in lactic acidosis has already started while intracellular pH remains constant. So, pH\(_T\) does not reflect the threshold of onset of glycolytic energy metabolism. In the report of Systrom et al. (1990), blood for La measurement was sampled from antecubital vein, though \(^{31}\)P-MRS was obtained from calf muscle during planter flexion. And, each measurement, blood La or \(^{31}\)P-MRS, was done in a separate exercise of experiment in the same exercise protocol. It might not be difficult to consider that there was some time delay between La production in the calf muscle and detection of La in the venous blood sampled from upper limb, especially in the case of exercise which did not recruit large muscle groups and enhance the cardio-circulatory systems strongly. In the present study, the time delay could be ignored if any, because \(^{31}\)P-MRS and blood La were measured from the same upper limb simultaneously.

Chwalbinska-Moneta et al. (1989) reported in their study by using muscle biopsy technique that blood LT appeared just after muscle LT, but muscle (H\(^+\)) was kept at resting level whenever muscle and blood La increased. One of the causes of stability of (H\(^+\)) which they discussed was an intermittent exercise protocol used in their study. The results of their study agree with that of the present study, although it is clear that intermittent exercise is not the cause of muscle pH which remained constant.

The smaller pH\(_T\) value of 43.0 % of WR\text{max} in the present study than that of 66.4 % of VO\(_2\text{max}\) by Systrom et al. (1990) and 61 % of WR\text{max} by Marsh et al. (1991) could not be explained. There might be some unknown difference in physical characteristics of the population tested. It is well known that AT obtained during cycling or running in untrained subjects is about 60 % of VO\(_2\text{max}\) (Fukuba, 1986). Davis et al. (1976), however, reported that AT determined by gas exchange parameters during arm exercise was smaller than that during leg exercise because of differences in training effect by daily activity, recruitment patterns of motor units and muscle fiber composition. So, it could be considered that LT of 24.8 % of WR\text{max} by wrist flexion in the present study to be reasonable.

It is considered that physiological meaning of pH\(_T\) is not the onset of lactate production due to glycolysis, but the work level at which the intracel-
inal acid-base equilibrium could not remain constant level against lactic acidosis during incremental exercise. It has been reported that intracellular acid-base equilibrium is maintained by buffer of PCr hydrolysis (Adams et al., 1990), (HCO$_3^-$) (Beaver et al., 1986; Hood et al., 1988), (HPO$_4^{2-}$) (Chasiotis, 1983; Adams et al., 1990) and some proteins (Parkhouse and McKenzie, 1984). Lactic acidosis existed at a low contraction level before pHTr is possible with reference to some reports that suggest glycolytic energy metabolism does not always reflect the hypoxic conditions of the muscle tissue (Ivy et al., 1981; Saltin et al., 1976; Keul et al., 1967; Jobsis, 1963; Connell et al., 1984).

Another possible mechanism to explain the earlier LT than pHTr is a difference in efflux rates between (H$^+$) and La from muscle into extracellular space. Benade and Heisler (1978) demonstrated that (H$^+$) showed a larger rate of efflux than that of La in vitro. In addition, some authors discussed that extracellular concentration of (HCO$_3^-$) affects the intracellular (H$^+$) removal (Costill et al., 1984; Hood et al., 1988; McNaughton et al., 1991; Wilkes et al., 1983). However, extracellular acid-base balance was not measured in the present study, and further experiments will be needed to study the relationship between pHTr and extracellular acid-base equilibrium.

Marsh et al. (1991) demonstrated that pHTr coincided with PT, and discussed that PT reflects the intracellular phosphorylation potentials of muscle. In this study, significant correlation between pHTr and PT was also obtained. Sahlin (1986) described that increased concentration of (H$^+$) will displace the creatine kinase equilibrium towards PCr breakdown, and high concentration of (H$^+$) per se did not limit muscular force generation, but a decrease in PCr did. The result of significant correlation between pHTr and PT by Marsh et al. (1991) and the present study could be speculated to support the above hypothesis. However, Wilson et al. (1988) discussed that muscle fatigue was caused by an increase in Pi (H$_2$PO$_4^-$) rather than by a decrease in intramuscular pH. It is difficult to explain the relationship between pHTr and PT inductively, because PT is determined by the change in Pi/PCr affected by both changes in Pi and PCr.

In conclusion, pHTr does not reflect the threshold of La production in muscles, and biochemical statement of working muscle with special reference to acid-base balance is kept well for a time after LT.

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