Tohoku J. Exp. Med., 2006, **208**, 307-320

Contribution of Intramuscular Oxidative Metabolism to Total ATP Production during Forearm Isometric Exercise at Varying Intensities

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Kimura, N., Hamaoka, T., Kurosawa, Y. and Katsumura, T. *Contribution of Intramuscular Oxidative Metabolism to Total ATP Production during Forearm Isometric Exercise at Varying Intensities*. Tohoku J. Exp. Med., 2006, **208** (4), 307-320 ── It is not fully clear whether intramuscular oxidative metabolism contributes to total adenosine triphosphate (ATP) production during forearm isometric exercise at varying intensities. We tested hypothesis that oxidative metabolism with intramuscular O_2 contributes to lessen the dependence on anaerobic metabolism, in particular phosphocreatine (PCr) breakdown. Seven male subjects were tested for changes in muscle oxygenation (MO_2) and high-energy phosphates in forearm flexor muscles at rest and during exercise under arterial occlusion by 31-phosphorus magnetic resonance spectroscopy (^{31}P-MRS) and near infrared spectroscopy (NIRS). Isometric wrist flexion exercise was performed for 1 min or until exhaustion at intensities corresponding to 30%, 50% and 70% of maximal voluntary contraction (MVC) under intramuscular O_2 (Intramuscular O_2-Ex) and anaerobic (Anaero-Ex) conditions. Oxidative ATP production in Intramuscular O_2-Ex was calculated as 0.05 ± 0.01 mM/s for 30%MVC, 0.08 ± 0.01 mM/s for 50%MVC and 0.11 ± 0.01 mM/s for 70%MVC. At a lower intensity (30%MVC), PCr breakdown rate (0.17 ± 0.02 mM/s) of Anaero-Ex was significantly higher than the rate (0.13 ± 0.01 mM/s) of Intramuscular O_2-Ex ($p < 0.05$). There was no significant difference in ATP production rates through PCr breakdown and glycolysis between Intramuscular O_2-Ex and Anaero-Ex at the higher intensities (50% and 70%MVC). In conclusion, intramuscular oxidative metabolism plays a significant role in reducing the dependence on PCr breakdown during isometric exercise at a lower intensity (30%MVC). ──── intramuscular O_2; oxidative metabolism; PCr breakdown
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Received August 29, 2005; revision accepted for publication February 9, 2006.
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The O_2 availability (oxidative metabolism) in the muscle during exercise is influenced by the O_2 supply from the arterial blood flow and the mitochondrial oxidative capacity. The decrease of O_2 supply to contracting muscle restricts adenosine triphosphase (ATP) production throughout oxidative phospholyration. Several exercise factors such as exercise type (continuous and intermittent), intensity, frequency and duration affect the O_2 supply from arterial blood. In particular, an important factor affecting O_2 supply during continuous contraction (isometric exercise) is the intramuscular pressure (IMP)(Sadamoto et al. 1983; Jarvholm et al. 1988; Aratow et al. 1993). The increase of IMP begins to compress intramuscular blood vessels and finally reaches a critical level that occludes the arterial blood flow. In previous studies, O_2 supply from the arterial blood flow was affected by the mechanical compression above approximately 30% of maximal voluntary contraction (MVC) and completely occluded above 40%MVC (Zwarts and Nielsen 1988). Therefore, isometric exercise at a higher intensity is similar to ischemic conditions under the restriction of O_2 supply. As O_2 supply is arrested completely, mitochondrial activity (respiration) does not stop immediately. Mitochondria can utilize the intramuscular O_2 in the muscle. The magnitude of intramuscular O_2 availability is determined by the mitochondrial oxidative capacity. Previous studies have reported that resting O_2 intramuscular in forearm muscles ranged on average from 0.34 to 0.45 mM (Blei et al. 1993; Hamaoka et al. 1996). This availability of intramuscular O_2 in the muscle may contribute to the energy demand during muscle contraction until O_2 depletion is achieved.

In this study, we performed isometric exercise at varying intensities under intramuscular O_2 and anaerobic conditions. The ATP is continuously resynthesized from phosphocreatine (PCr) during aerobic exercise. And, this PCr is resynthesized from creatine (Cr) by oxidative metabolism in mitochondria. It is speculated that oxidative metabolism with intramuscular O_2 will contribute to lessen the dependence on anaerobic metabolism, in particular PCr breakdown, during exercise at lower intensity. With an increase in exercise intensity, type II or fast twitch fibers in muscle mainly begin to recruit. At that point, the contribution of anaerobic metabolism (PCr breakdown and/or glycolysis) during isometric exercise is enhanced. And, relative contribution of oxidative metabolism at higher intensity is less than that at lower intensity. Oxidative metabolism is affected by several metabolic factors such as adenosine diphosphate (ADP) and acidosis. The rise in ADP lies in its role in activating oxidative phosphorylation (Walter et al. 1997; Conley et al. 2001). In contrast, acidosis resulting from the accumulation of H^+ provokes the reduction in sending a signal for oxidative phosphorylation, and affects mitochondrial oxidative capacity (Harkema and Meyer 1997; Walsh et al. 2002). We hypothesize that, due to the larger ATP cost for continuous contraction at higher intensity, the increase in anaerobic metabolism and/or acidosis may attenuate the effect of rise in ADP on mitochondrial O_2 availability. Therefore, the contribution of oxidative metabolism with intramuscular O_2 at higher intensity to the decreasing of PCr breakdown may be much smaller.

The purpose of this study was to examine the availability of intramuscular O_2 in forearm muscles during isometric exercise at varying intensities and to quantify the contribution of intramuscular oxidative metabolism to total ATP production, in particular PCr breakdown. We tested hypothesis that oxidative metabolism with intramuscular O_2 contributes to lessen the dependence on anaerobic metabolism, in particular PCr breakdown.

Methods

Subjects

Seven healthy male subjects (ranging in age from 24 to 30 years) participated in this study. All subjects voluntarily gave written informed consent to participate in this protocol in accordance with the ethical committee of university (Ethical Committee on Research Intended for Human Subject in Nippon Sport Science University). Their height, weight and grip strength were 173.3 ± 4.1 (mean ± S.E.) cm, 66.8 ± 4.2 kg, 52.1 ± 4.0 (dominant) kg and 48.3 ± 5.2 (non-dominant) kg, respectively. The

forearm flexor muscles, mainly the flexor digitorum superficialis muscles, of the right arm in all subjects were used as the measurement site.

Experiment protocol

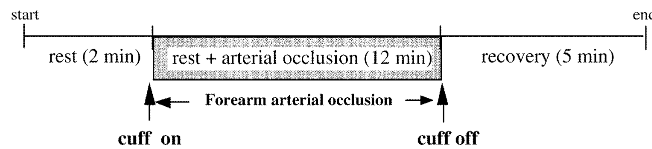
Three experimental protocols (Fig. 1) were performed on each subject: the measurements of 1) resting metabolic rate (Rest), 2) exercise under intramuscular O_2 condition (Intramuscular O_2-Ex) and 3) exercise under anaerobic condition (Anaero-Ex). Continuous changes in energy metabolism (PCr, Pi and intracellular pH) and MO_2 kinetics in the muscle were measured by 31-phosphorus magnetic resonance spectroscopy (^{31}P-MRS) and near infrared spectroscopy (NIRS), separately.

The resting metabolic rate was determined using the arterial occlusion method. This method has been extensively demonstrated in previous studies (Hampson and Piantadosi 1988; Hamaoka et al. 1996). After a 2 min rest period, forearm arterial blood flow was arrested by placing a pneumatic cuff on the upper arm at a pressure of 280 mmHg. Arterial occlusion was maintained for 12 min at rest. During a 12 min arterial occlusion, the rate of muscle oxygenation (MO_2) decline measured by NIRS is an indicator of aerobic resting metabolism. The kinetic of PCr breakdown was also measured with ^{31}P-MRS. This rate of PCr breakdown after O_2-depletion is an indicator of anaerobic resting metabolism (Hamaoka et al. 1996).

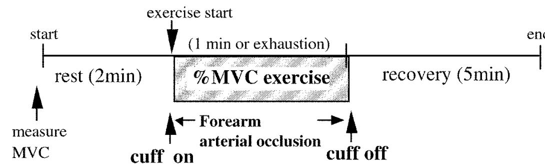
Isometric wrist flexion exercise (Intramuscular O_2 -Ex and Anaero-Ex) was performed at a constant force corresponding to 30%, 50% and 70% of maximal voluntary contraction (MVC). The duration of each exercise was 1 min for 30% and 50%MVC, and until exhaustion for 70%MVC. Intramuscular O_2 -Ex was performed just after arterial occlusion by a pneumatic cuff. In the case of Intramuscular O_2 -Ex, forearm blood flow was blocked by a pneumatic cuff just before the start of each %MVC exercise. Then, the mitochondria consumed only the O_2 stored in the muscle until O_2 depletion. Therefore, the rates of MO_2 decline during

Experimental Protocol

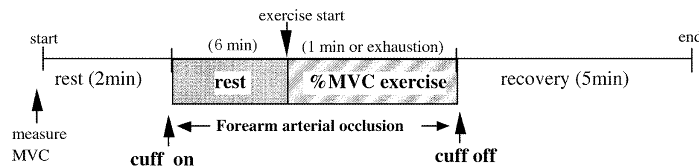
Resting metabolic rate (Rest)



Intramuscular O_2 Exercise (Intramuscular O_2-Ex)



Anaerobic Exercise (Anaero-Ex)



cuff on; **a pneumatic cuff on the upper arm at a pressure of 280 mmHg**

; Isometric wrist flexion exercise at 30%, 50% (for 1 min) and 70%MVC (until exhaustion).

Fig. 1. Experimental design for each protocol.

Intramuscular O_2-Ex were considered to be indicative of the intramuscular O_2 availability in muscle at all intensities. An anaerobic condition in muscle was created by applying arterial occlusion at rest. This occlusion was performed until O_2 was depleted (minimum oxygenation level) in the muscle. The duration until O_2 depletion in the muscle was complete ranged from 5 to 6 min, and O_2 depletion was confirmed by measuring the resting metabolic rate using NIRS. After O_2 depletion, Anaero-Ex was performed under O_2 depleted condition. In both Intramuscular O_2 -Ex and Anaero-Ex, the pneumatic cuff was deflated immediately after isometric exercise, and the subjects remained in the sitting position for 5 min post-exercise.

Each measurement (Rest, Intramuscular O_2-Ex and Anaero-Ex) was taken twice separated by a minimum 3-day interval: once with the ^{31}P-MRS in the magnet and once with the NIRS outside the magnet, since the NIRS system would be influenced by the presence of a strong magnetic field. Thus, the measurements were conducted randomly 14 times (12 measurements as the exercise protocol (2 protocols [Intramuscular O_2-Ex and Anaero-Ex]) at 3 intensities (30, 50, 70%MVC) at twice (NIRS and ^{31}P-MRS) and 2 measurements as the resting protocol (NIRS and ^{31}P-MRS) on the 14 different days for each subject.

Fig. 2 shows the essential parts of experimental set-up for exercise protocol. In the sitting position, each subject's right hand was attached to the surface coil (^{31}P-MRS) on the platform, and the probe (NIRS) was strapped on to the forearm flexor muscles. Isometric wrist flexion exercise was performed using a wrist flexion ergometer with strain-gauge force transducers. In the sitting position using the right hand, wrist angle of each subject was adjusted to between 0 to 10°. First, wrist flexion exercises of MVC of over 1 s were performed three times separated by 1 min by all of the subjects. The highest individual value of three trials was taken as the MVC of the subject. After several minutes of rest, each subject performed the isometric wrist flexion exercise for 1 min at 30% and 50%MVC, and until exhaustion at 70%MVC. The strength necessary for the required %MVC was marked on the dynamometer for visual feedback to the subject. During isometric exercise, the subject maintained the required strength looking at the indicator on the dynamometer (Fig. 2) to sustain the prescribed force.

^{31}P-magnetic resonance spectroscopy

^{31}P-spectra were collected with the nuclear magnetic resonance (NMR) (Otsuka Electronic Co. Ltd., Osaka) using a 2.0 -T, 26 cm horizontal-bore magnet. The NMR spectra were obtained from a 3 cm diameter circular two-tune surface coil placed over the forearm flexor muscles. After being inserted into the superconducting magnet, a surface coil on the platform was located in the center of the magnetic field and tuned to frequencies of 43.58 Hz for ^{31}P and 89.57 Hz for ^{1}H. Homogeneity of the magnetic field was optimized by shimming (adjusted to < 0.30 ppm in the typical line width at half height) on the proton

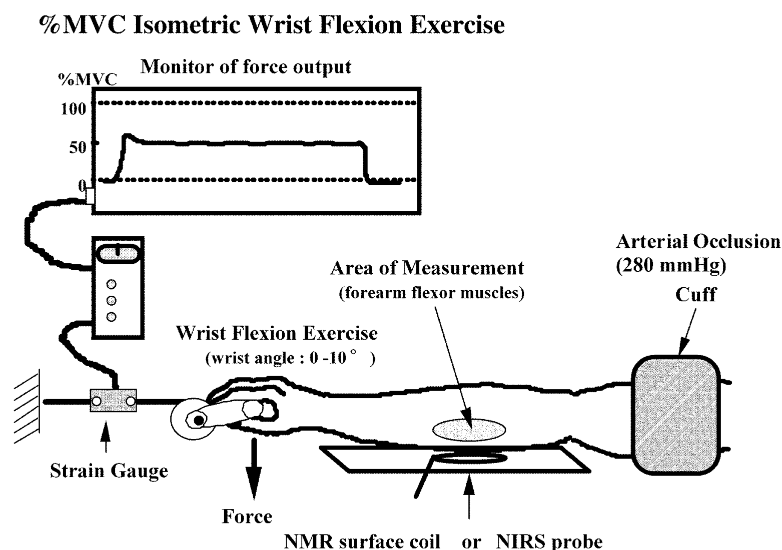


Fig. 2. Schematic presentation of experimental set-up.

signal from the water. After switching to ^{31}P, the receiver gain for ^{31}P was set to maximize the PCr signal acquired from the muscle, and kept at an adequate level throughout the experiment. The continuous Free Induction Decays (FIDs) were acquired with a repetition time of 2 s throughout this experiment. The NMR spectrum was collected every 60 s during rest using a spectrum (average of 30 FIDs) and every 20 s (average of 10 FIDs) during exercise. The FIDs parameters were set with the flip angle at 90°, pulse width at 60, spectral width at 5,000 Hz and 1,024 sampled FIDs.

^{31}P-spectra were processed with an exponential line broadening equivalent to 5 Hz to improve the signal to noise ratio after Fourier transformation. The corrected baseline and areas of inorganic phosphate (Pi), PCr and β-ATP peaks were fitted by using the least-squares method including each area and frequency of the individual peaks. The peak areas were corrected for possible saturation effects. The variation in concentration of Pi, PCr and β-ATP were expressed relative to the sum of the total phosphorus (T): T = Pi + PCr. Absolute concentration of each parameter was calculated from the resting β-ATP concentration of 8.2 mM (Harris et al. 1974; Henriksson et al. 1986). Intracellular pH was calculated from the chemical shift between Pi and PCr by using the following equation; pH = 6.73 + log 10 ([@- 3.275]/[5.685 -@]) where @ is the chemical shift from Pi to PCr (Kushmerick and Meyer 1985). ADP concentration was also calculated from intracellular pH and PCr using total creatine (TCr; 42 mM) measured at each time point; ADP = ([ATP] × [TCr])/([PCr] × $[H^+]$ × [Keq]) where Keq is equilibrium constant of the creatine kinase reaction ($1.66 \times 10^9 M^{-1}$) (Sahlin et al. 1975).

NIRS

NIRS (HEO- 200, Omron Co. Ltd., Kyoto) used for this study consisted of a probe (4.0 × 7.0 × 2.0 cm and weight 85 g) and a computerized control segment. The wavelengths of the two light sources in this probe were 760 and 840 nm, and the distance between the light sources and the detector was 3 cm. With adequate signal intensity, the light penetration depth was 1 - 2 cm under the skin surface. Continuous signal changes in oxygenation of hemoglobin (Hb) and/or myoglobin (Mb) and blood volume (Total Hb and/or Mb) were monitored every second (Shiga et al. 1995), and that data was intramuscular in a computer system. The MO_2 and blood volume were normalized to the overall changes, and MO_2 in the tissues was expressed as a relative value. The percent of MO_2 change was calculated by defining resting MO_2 as 100% and MO_2 depletion during arterial occlusion as 0% (Hamaoka et al. 1996). Blood volume was also corrected by fat layer thickness on forearm flexor muscles (Niwayama et al. 2000).

Analysis of NIRS and ^{31}P-MRS data

The change in MO_2 and blood volume by NIRS in one subject at 50%MVC exercise is shown in Fig. 3. From the start of Intramuscular O_2-Ex, MO_2 showed a linear decline (phase with an adequate O_2 availability). After approximately 20 s, the rate of MO_2 decline attenuated. Thereafter, MO_2 reached a plateau or only a slight decline (phase with a restricted O_2 availability). From the change in MO_2 during Intramuscular O_2-Ex, the rate of MO_2 decline and the minimum level of MO_2 change (A-point) were calculated as follows. A-point is the lowest level of MO_2 change during Intramuscular O_2-Ex at all intensities. This A-point was determined from the average of three points in the minimum level. And, we calculated the consumed $\%MO_2$ (delta % MO_2: resting % MO_2 - $\%MO_2$ at A-point) during Intramuscular O_2-Ex at each %MVC. The rate of MO_2 decline during Intramuscular O_2-Ex was plotted every 1 s and a linear regression analysis was applied to these data until A-point. The slope of the best-fit line every 5 s was calculated the correlation coefficients in an individual subject ranged from $r = -0.90$ to $r = -0.99$. The peak slope for 5 s, the slope every 5 s and MO_2 level at A-point in an individual subject were estimated to be indicative of intramuscular O_2 availability. The rates of PCr breakdown, Pi increase and intracellular pH change during isometric exercises were also determined by the slope of a linear regression analysis of concentration vs. time (three or four data points). The changes for each parameter were plotted, and the slopes were calculated from the single regression providing the best-fit curve of the data. The ATP production rate (ATP mM/s) during both exercises until A-point was reached was determined by these parameters from both ^{31}P-MRS and NIRS.

The oxidative ATP production (Q) was determined using both of the metabolic rates measured during Intramuscular O_2-Ex and at rest, as previously described (Hamaoka et al. 1996; Kimura et al. 2004). Assuming that the resting metabolic rate did not change during the 12 min under arterial occlusion, ATP production rate by oxidative phosphorylation (the rate of aerobic metabolism) should be equivalent to the rate of PCr breakdown after MO_2 depletion (the rate of anaerobic metabolism).

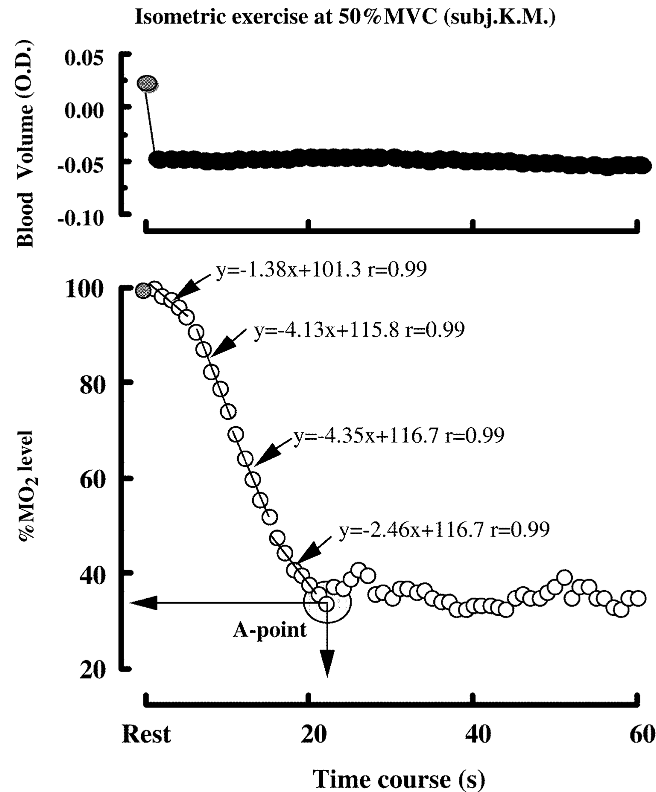


Fig. 3. Schematic presentation of the calculation in MO_2 decline rate and A-point level
Slope: the slope of linear regression analysis every 5 s.
A-point: the minimum level (3 s points averaged) in MO_2 change during intramuscular O_2-Ex.

In this study, the rate of MO_2 decline during exercise was expressed relative to that of the resting values. We also multiplied the ratio by PCr breakdown rate at rest. The oxidative ATP production (Q) was calculated as follows (Kimura et al. 2004):

$$Q \text{ (mM/s ATP)} = \frac{MO_2 \text{ decline rate (exercise)}}{MO_2 \text{ decline rate (rest)}} \times \text{PCr breakdown rate (rest)}$$

Where MO_2 decline rate (exercise) is the average rate of MO_2 decline during the aerobic phase of Intramuscular O_2-Ex, MO_2 decline rate (rest) is the rate of MO_2 decline during arterial occlusion at rest and PCr breakdown rate (rest) is the rate of PCr breakdown at rest after complete MO_2 depletion.

ATP production by PCr hydrolysis (D) was determined by the rate of PCr breakdown during isometric exercises under two conditions.

Glycolytic ATP production (L) was determined from the metabolic proton production during isometric exercise (Kemp et al. 1993, 1994; Kemp and Radda 1994; Kemp et al. 1996; Kimura et al. 2004). We calculated the metabolic proton production from the amount of protons consumed by PCr hydrolysis(ϕ), buffered passively in the cytosol (β: buffer capacity) and produced by oxidative ATP (mQ):

$$L = 1.5 \times (-\beta \times \text{[the rate of pH change]} - mQ - \phi \times \text{[the rate of PCr breakdown]}),$$

Where the creatine kinase reaction consumes ϕ protons per PCr used to produce ATP. The ϕ is net proton stoichiometry per PCr. The "mQ" is the contribution of oxidative ATP production to changes in proton load. The buffer capacity (β) in the muscle is defined as the amount of protons needed to decrease the pH of 1 L of muscle by one pH unit. The protons are buffered in the muscle by Pi (β[Pi]), bicarbonate (β[bi-]) and non bicarbonate/non-Pi (e.g. protein; [β(non-Pi/non-b)]). The buffer capacity values used in this study is the sum of these components, and the principles of these calculations are discussed in previous published studies (Walter et al.

1999; Kimura et al. 2004). By combining these proton stoichiometries and the buffer capacity at any time point during exercise, it was possible to calculate the glycolytic ATP production based on changes in intracellular pH. We also multiplied the sum of the calculated proton production by 1.5 (the glycolysis generation of 1 mole ATP generates 2/3 mole lactic acid).

Statistics

All data are presented as mean ± S.E. Relationships between each parameter were determined using a linear regression analysis. In each experiment, comparisons of the data were made using a two-way analysis of variance (ANOVA). When a significant F ratio was found, Bonferoni/Dunn post hoc test was employed to locate difference between specific means. A statistical significance was accepted at $p < 0.05$.

Results

From the onset of exercise at all %MVC, MO_2 showed a linear decline in the initial phase, the duration of which varied in all intensities (Fig. 4). The rates of MO_2 decline were elevated with an increase in exercise intensity. Thereafter, the rate of MO_2 decline began to attenuate and changed into a plateau or only a slight decline. Blood volume fell immediately after the start of each isometric exercise and, after ranging from 2 to 4 s, maintained a constant level. In the changes of NIRS data during Anaero-Ex, MO_2 fell slightly immediately after the start of each %MVC exercise after O_2 depletion. Thereafter, MO_2 remained constant near the minimum level (near 0%MO_2 level) during all Anaero-Ex. From this result, we confirmed that MO_2 was not utilized after O_2 depletion.

Fig. 5 shows the changes in MO_2 decline rate every 5 s and peak rate for 5 s during Intramuscular O_2-Ex at three intensities. For the initial 10 s of isometric exercise, MO_2 decline rate at each intensity showed the highest values, and elevated with an increase in exercise intensity. Thereafter, the rates of MO_2 decline at all intensities began to

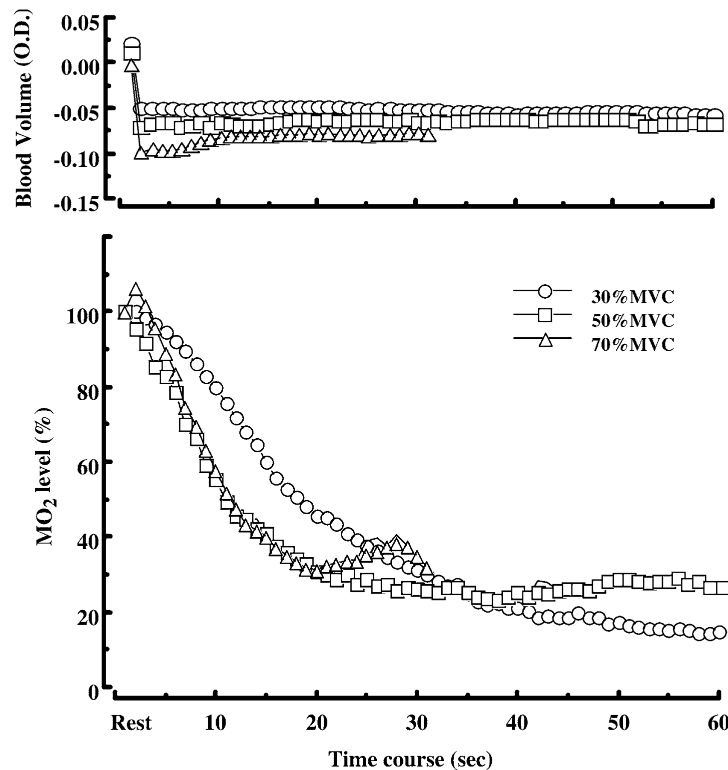


Fig. 4. The kinetics of muscle oxygenation (MO_2) and blood volume (B.V.) during Intramuscular O_2-Ex at varying intensities.

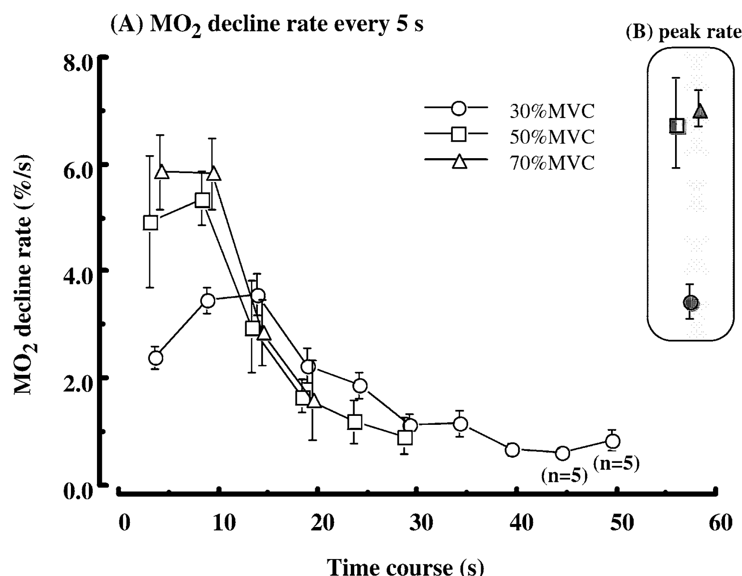


Fig. 5. Changes in MO_2 decline rate every 5 s (A) and peak rate (B) during intramuscular O_2-Ex at varying intensities.
Peak rate: the peak rate of MO_2 decline for 5 s during intramuscular O_2-Ex.

attenuate, in particular, the rate in both 50% and 70%MVC decreased sharply. In contrast, the rate of 30%MVC declined more slowly during Intramuscular O_2-Ex. The peak rates of MO_2 decline were 3.69 ± 0.28 %/s (30%), 6.83 ± 0.78 %/s (50%) and 7.00 ± 0.18 %/s (70%). There was no significant difference in peak rates of MO_2 decline for 5 s between 50% and 70%MVC.

Fig. 6 compares the changes in PCr, Pi and intracellular pH during both exercises at each %MVC. There was no significant difference in either PCr or intracellular pH between each "Rest" and "Rest1" of Anaero-Ex. From the onset of each exercise, PCr fell and Pi rose linearly with time. The rates of PCr breakdown and Pi increase in each exercise were elevated with an increase in exercise intensity. In lower intensity (30%MVC), PCr breakdown rate in Anaero-Ex was higher than the rate of Intramuscular O_2-Ex. However, in the highest intensity (70%MVC), no significant differences were found in PCr breakdown or Pi increase between Intramuscular O_2-Ex and Anaero-Ex. During the initial phase (10 s) of each %MVC exercise, intracellular pH rose slightly, thereafter, intracellular pH in all intensities showed a linear decline with time. The rate of intracellular pH change at 30%MVC was slower and the rate at 70%MVC was more rapid. However, there were no significant difference in intracellular pH at each %MVC between Intramuscular O_2-Ex and Anaero-Ex.

From the start, the calculated ADP level during Intramuscular O_2-Ex at each %MVC increased gradually with time, and also elevated with an increase in exercise intensity (Fig. 7). ADP level at exhaustion (70%MVC) and 50 s (30% and 50%MVC) for Intramuscular O_2-Ex are as follows: 37.4 ± 2.0 μM (30%), 54.6 ± 8.1 μM (50%) and 112.2 ± 26.7 μM (70%) for Intramuscular O_2-Ex.

The average of MO_2 decline rate (A) and consumed $\%MO_2$ until A-point (B), ADP at A-point (C) and intracellular pH at Rest and at A-point (D) were calculated during Intramuscular O_2-Ex at three intensities Fig. 8. The rate of MO_2 decline at rest was 0.36 ± 0.06%/s. The average rates of MO_2 decline during Intramuscular O_2-Ex were calculated as 1.79 ± 0.14%/s (30%), 2.91 ± 0.27%/s (50%) and 3.68 ± 0.39%/s (70%) ($p < 0.05$), respectively. However, no significant difference in the rate of MO_2 decline was found between 50% and 70%MVC. Consumed $\%MO_2$

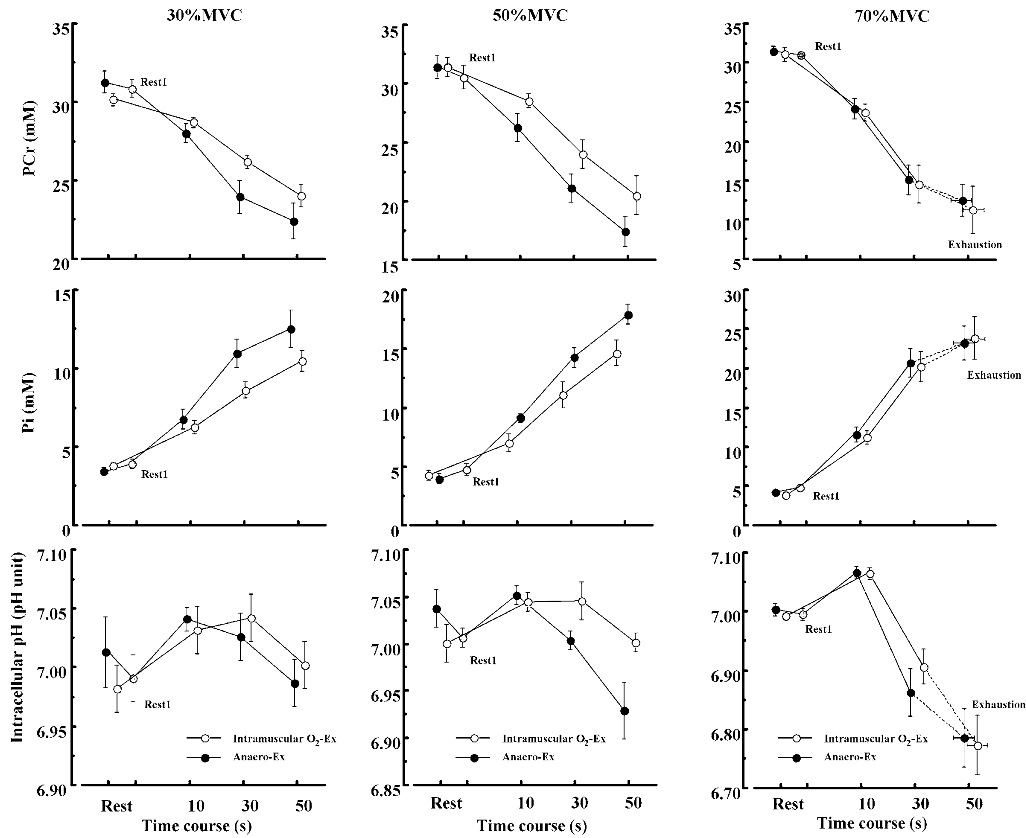


Fig. 6. Changes in phosphocreatine (PCr), Pi and intracellular pH during two isometric exercises (Intramuscular O_2-Ex and Anaero-Ex) at three intensities.
The plotted "Rest1" represents the time point at rest under arterial occlusion immediately before the onset of exercise in anaerobic conditions (Anaero-Ex).

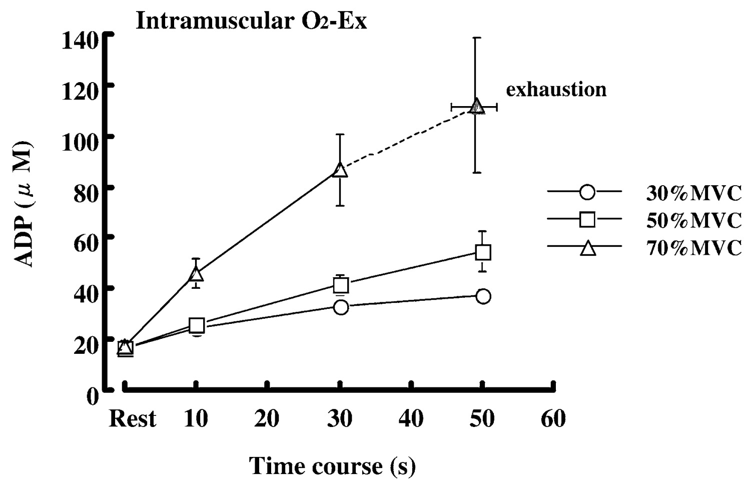


Fig. 7. Change in calculated ADP during Intramuscular O_2-Ex at varying intensities.

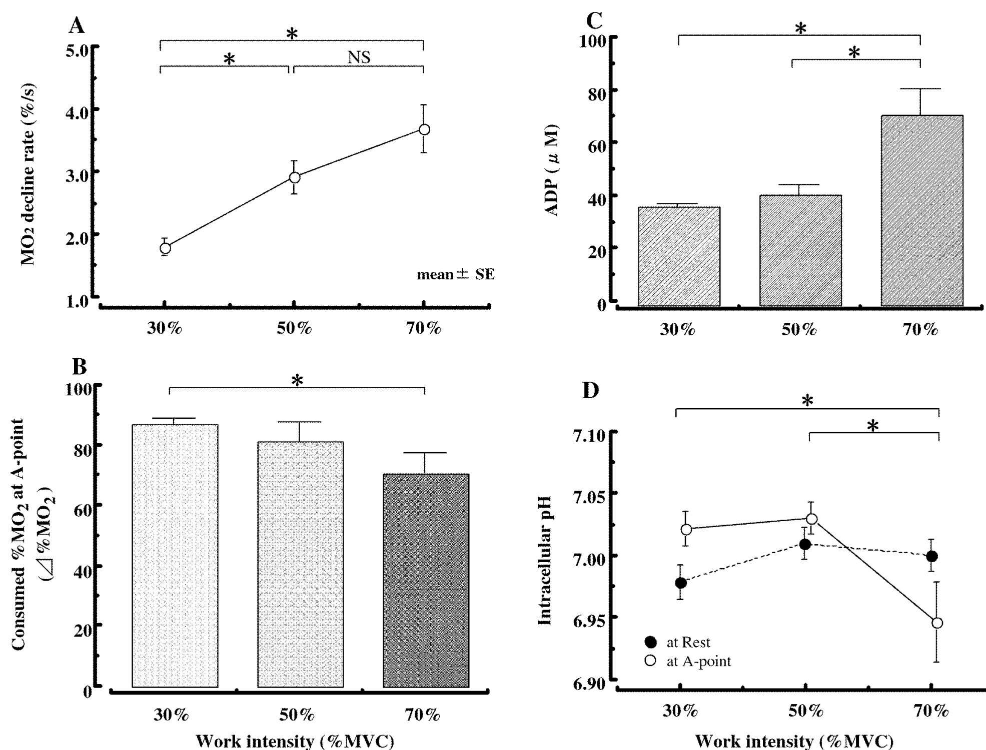


Fig. 8. The average rates of MO_2 decline (A) and consumed $\%MO_2$ until A-point (B), ADP at A-point (C) and intracellular pH at rest and at A-point (D) during Intramuscular O_2-Ex at three intensities.
A: The average of MO_2 decline rate every 5 s until A-point.
B: consumed $\%MO_2$ = (resting % MO_2 - $\%MO_2$ at A-point).
C: The calculated ADP level at A-point.
D: intracellular pH at rest and at A-point.
$^*p < 0.05$; Significant difference among three intensities (A, B, C).
$^*p < 0.05$; Significant difference in intracellular pH at A-point among three intensities (D).

until A-point was 86.8 ± 2.1% (30%), 81.4 ± 6.0% (50%) and 70.4 ± 6.8% (70%), respectively. There was a significant difference ($p < 0.05$) in consumed $\%MO_2$ between 30% and 70%MVC. ADP level at A-point for 70%MVC was also significantly higher ($p < 0.05$) than that of both 30% and 50%MVC. Intracellular pH at A-point was 7.02 ± 0.02 pH unit, 7.03 ± 0.01 pH unit and 6.93 ± 0.03 pH unit for 30%, 50% and 70%MVC, respectively. Intracellular pH at A-point for 70%MVC was significantly lower ($p < 0.05$) than that of other intensities (30% and 50%MVC).

ATP production rate during isometric wrist flexion exercise at varying intensities under two conditions is shown in Fig. 9. The resting metabolic rate was 0.01 ± 0.001 mM/s. Each rate of ATP production during exercise under two conditions was elevated with an increase in exercise intensity. There was no significant difference in total ATP production rate (oxidation + PCr breakdown + glycolysis) between Intramuscular O_2-Ex and Anaero-Ex. Oxidative ATP production in Intramuscular O_2-Ex was 0.05 ± 0.01 mM/s, 0.08 ± 0.01 mM/s and 0.11 ± 0.01 mM/s for 30%, 50% and 70%MVC, respectively. In lower intensity (30%MVC), PCr breakdown rate (0.17 ± 0.02 mM/s) of Anaero-Ex was higher than the rate (0.13 ± 0.01 mM/s) of Intramuscular O_2-Ex (< 0.05). In contrast, there were no significant differences in ATP production rates through PCr breakdown and glycolysis between Intramuscular O_2-Ex and Anaero-Ex at the higher intensities (0.24 ± 0.04 mM/s vs 0.32 ± 0.03 mM/s for 50% and 0.59 ± 0.06 mM/s vs 0.56 ± 0.06 mM/s for 70%MVC in

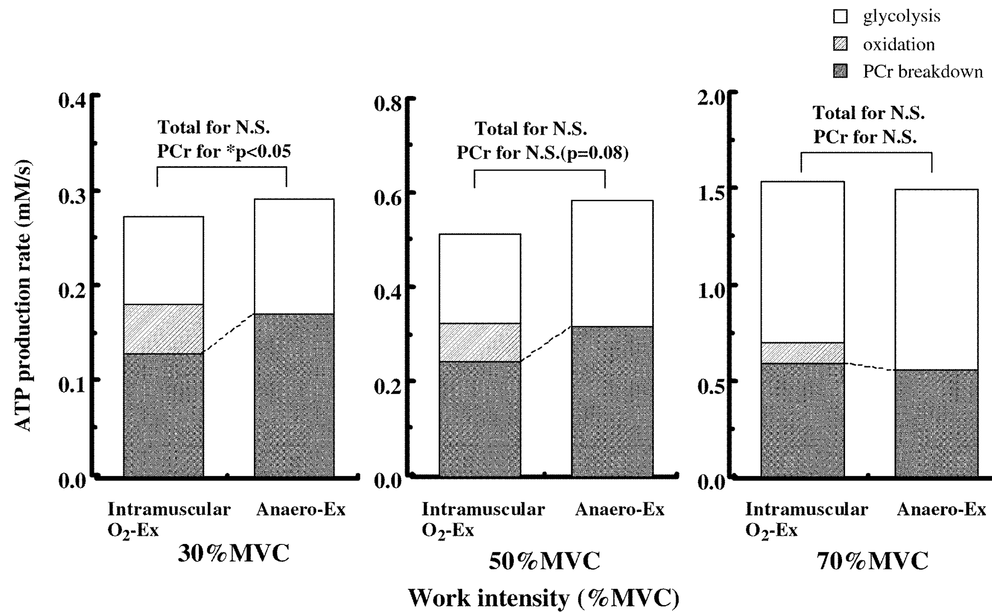


Fig. 9. Changes in ATP production rates (mM/s) during isometric exercise at varying intensities under each condition.
$^{*}p < 0.05$; Intramuscular O_2-Ex vs Anaero-Ex for phosphocreatine (PCr) breakdown.
N S; Intramuscular O_2-Ex vs Anaero-Ex for the sum of ATP productin.

PCr breakdown, 0.15 ± 0.04 mM/s vs 0.27 ± 0.05 mM/s for 50% and 0.84 ± 0.16 mM/s vs 0.93 ± 0.21 mM/s for 70%MVC in glycolysis, respectively).

Discussion

We estimated the contribution of intramuscular oxidative metabolism to total ATP production during isometric exercise at varying intensities. At a lower intensity (30%MVC), anaerobic metabolism, in particular PCr breakdown of Intramuscular O_2-Ex was significantly lower ($p < 0.05$) than that of Anaero-Ex. This result suggests that oxidative metabolism with intramuscular O_2 plays a significant role in reducing dependence on anaerobic metabolism during isometric exercise at a lower intensity. However, oxidative metabolism using intramuscular O_2 above 50%MVC did not contribute to the decrease in PCr breakdown.

The contribution of intramuscular oxidative metabolism to total ATP production during isometric exercise at lower intensity

We performed the arterial occlusion by cuff immediately before the start of each %MVC exercise. As O_2 supply from arterial blood flow was arrested, mitochondria utilized only the intramuscular O_2 in the muscle. Therefore, the availability of intramuscular O_2 under arterial occlusion is an index of O_2 consumption (VO_2) in required energy demand of each %MVC exercise. And, the magnitude of intramuscular O_2 availability is determined by the mitochondrial oxidative capacity (Hamaoka et al. 1996). In this study, MO_2 decline rate (peak and average) and percent MO_2 level at A-point were the indexes of the availability of intramuscular O_2 during %MVC exercise. From the start of each %MVC exercise, the availability of intramuscular O_2 showed a linear increase. In the initial 10 s of exercise at three intensities, the rates of intramuscular O_2 availability were the highest level, and were elevated with an increase in exercise intensity. The peak rate of each %MVC ranged from 10.2 (30%MVC) to 19.4 (70%MVC) times of resting metabolic rate. These results suggest that the availability of intramuscular O_2 during isometric exercise was immediately activated in all intensities.

The O_2 availability in mitochondria (oxidative phosphorylation) was affected by several metabolic factors such as ADP and Pi. ADP was an important metabolic factor that controlled oxidative phosphorylation. The rising ADP lies in its role in activating oxidative phosphorylation (Walter et al. 1997; Conley et al. 2001). The effect of ADP on the O_2 availability in mitochondria during Intramuscular O_2-Ex elevated with an increase in exercise intensity. In exercising human skeletal muscle, motor units of type I (slow-twitch) fibers are recruited slowly when low-grade exercise is performed. With an increase in exercise intensity, there is a progressive recruitment of type II (fast-twitch) fibers until all motor units have been recruited during high intensity exercise (Saltin and Gollnick 1983). From the metabolic specificity and the different behavior of muscle fibers, mitochondrial O_2 availability is also enhanced by this recruitment of type I fibers during exercise. In this study, the availability of intramuscular O_2 (A of Fig. 8) rose linearly until 50%MVC. Therefore, the availability of intramuscular O_2 until 50%MVC may be influenced by both a rise of ADP and progressive recruitment of type I fibers in forearm muscles.

A previous study reported that muscle activation (Blei et al. 1993) and contractile cost per twitch (Conley et al. 1998) were unchanged during muscle contraction under ischemic and aerobic conditions. In the human forearm muscle during isometric exercise at three intensities, there were no significant differences in the sum of ATP production rates between Intramuscular O_2-Ex and Anaero-Ex. From this result, it could see that ATP costs during isometric exercise at three intensities were similar between Intramuscular O_2-Ex and Anaero-Ex.

The oxidative ATP production using intramuscular O_2 during exercise affects the anaerobic metabolism, in particular PCr breakdown. The change in PCr during aerobic exercise is the balance of PCr consumption and resynthesis. The creatine liberated from PCr during exercise migrated into the mitochondria via the creatine phosphate shuttle. The creatine was then resynthesized to PCr using ATP produced from oxidative phosphorylation in mitochondria. During isometric exercise at a lower intensity (30%MVC), PCr breakdown rate of Intramuscular O_2-Ex was significantly lower ($p < 0.05$) than that of Anaero-Ex. This difference in PCr breakdown rate at a lower intensity may reflect the contribution of oxidative ATP production. This result suggests that oxidative ATP production during Intramuscular O_2-Ex, until MO_2 depletion is achieved, may contribute to maintaining the PCr level. In addition, PCr breakdown rate (0.17 mM/s) for Anaero-Ex was similar to PCr breakdown rate plus oxidative ATP production (0.18 mM/s) for Intramuscular O_2-Ex. This similarity suggests that oxidative ATP production using intramuscular O_2 may be the main source of PCr resynthesis during Intramuscular O_2-Ex at a lower intensity.

The availability of intramuscular O_2 during isometric exercise at higher intensities

There was no significant difference in MO_2 decline rate (A of Fig. 8) between 50% and 70%MVC. The rise in MO_2 decline rate was not found at a higher intensity (70%MVC). In addition, consumed %MO_2 until A-point for 70%MVC was smaller than that at other intensities (B of Fig. 7). The greater consumed %MO_2 until A-point would represent the higher magnitude of O_2 availability in mitochondria. Thus, the intramuscular O_2 at higher intensity (70%MVC) was not utilized in spite of the fact that the MO_2 was intramuscular adequately. From these results, we considered that the availability of intramuscular O_2 at higher intensities, in particular at 70%MVC, was restricted.

This elevation in ADP strongly activated oxidative phosphorylation. However, the availability of intramuscular O_2 during intramuscular O_2-Ex at higher intensity (70%MVC) was not maintained despite an increase in ADP level. This result apparently showed that the effect of a rise in ADP on mitochondrial oxidative phosphorylation was extremely attenuated so as not to activate oxidative metabolism. However, this result alone is insufficient to account for the restriction in effect of ADP. This restriction may be attributed to

the larger contribution of anaerobic metabolism at a higher intensity. In the case of higher intensity exercise, type II fibers using anaerobic metabolism as their main ATP source were primarily recruited. Thus, the contribution of anaerobic metabolism increased from the start of exercise at higher intensity. Due to larger ATP demand during continuous contraction at higher intensity, ATP production via anaerobic metabolism is quick and efficient. In contrast, ATP production using oxidative metabolism is inefficient because it must go through many steps. Therefore, the increase of anaerobic metabolism at higher intensity counteracted the effect of a rise in ADP on the mitochondrial O_2 availability to permit the exercise to continue.

Another possibility is that the metabolic factor limiting the availability of intramuscular O_2 may be acidosis resulting from the accumulation of H^+. This acidosis (decrease in intracellular pH) affects the oxidative ATP production directly and/or indirectly. Previous studies have reported that intracellular acidosis indirectly affected the reduction in sending a signal for oxidative phosphorylation (such as ADP or phosphorylation potential, [ATP]/[ATP][Pi]) (Tonkonogi and Sahlin 1999; Conley et al. 2001). It has been demonstrated in several previous studies that on direct effect of acidosis are to reduce the oxidative capacity in mitochondria (isolated skeletal muscle fibers: Walsh et al. 2002; isolated cat muscle: Harkema and Meyer 1997).

In this study, intracellular pH of A-point at 70%MVC was significantly ($p < 0.05$) lower than that at 30% and 50%MVC. Intracellular pH level at 70%MVC was 6.93 ± 0.01 (ranging from 6.80 to 7.03). This is the upper level of intracellular acidosis (pH = 6.8) on oxidative phosphorylation as shown in previous studies (Conley et al. 2001; Jubrias et al. 2003). This result suggests that the effect of intracellular acidosis at 70%MVC was greater than at a lower %MVC. However, previous studies reported that relatively mild acidosis (pH 6.8-6.9) prevented a rise in oxidative flux despite a substantial rise in ADP (Jubrias et al. 2003). Thus, this mild acidosis at 70%MVC might also attenuate the availability of intramuscular O_2 in muscle.

Contribution of oxidative metabolism using intramuscular O_2 to total ATP production during isometric exercise at higher intensities

The relative contribution of oxidative ATP production using intramuscular O_2 on the sum of ATP production decreased with an increase in exercise intensity (19.0 [30%], 17.3 [50%] and 7.1 [70%] % of the sum of ATP production rate). In the case of exercise at higher intensity, type II or fast twitch fibers using glycolysis as the main ATP source were recruited from the start of the exercise. In this study, the required ATP sources for isometric contraction above 50%MVC strongly depended on anaerobic metabolism, in particular glycolysis. In addition, there was no significant difference in anaerobic metabolism (PCr breakdown) in either exercise above 50%MVC (Fig. 8). This result suggested that, with the larger ATP cost for continuous contraction at higher intensities, oxidative ATP production with intramuscular O_2 much lessens the contribution to PCr resynthesis during isometric exercise. We considered that the content of intramuscular O_2 (0.48 mM also as 2.9 mM ATP) in forearm muscles was much smaller than other anaerobic energy sources (PCr content at rest ranged from 31.1 to 31.6 mM). Therefore, with the larger ATP cost for continuous contraction at higher intensity, the contribution to PCr resynthesis by the oxidative metabolism through the remaining intramuscular O_2 may be counteracted. There is also the possibility that a part of the oxidative ATP production was directly used for the ATP expended during muscle contraction.

Conclusions

In conclusion, at a lower intensity (30%MVC), PCr breakdown rate of Intramuscular O_2-Ex was lower than the rate of Anaero-Ex. Therefore, intramuscular oxidative metabolism plays a significant role in reducing the dependence on PCr breakdown during isometric exercise at lower intensity. However, intramuscular oxidative metabolism above 50%MVC did not contribute to the decrease in PCr breakdown. In

addition, the availability of intramuscular O_2 at higher intensities was not enhanced in spite of the significantly increased ADP and adequate stored intramuscular O_2. Thus, at the higher intensity above 50%MVC, the increase of anaerobic metabolism and/or acidosis attenuated the effect of a rise in ADP on the availability of intramuscular O_2 in mitochondria.

Acknowledgments

We would like to thank the entire staff of the Department of Preventive Medicine and Public Health, Nippon Sport Science University, especially Takashi Ito, for excellent collaboration in this investigation.

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