Oscillation in Tissue Oxygen Index during Recovery from Exercise

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Running title: Oscillation of tissue oxygen index

Summary

It was hypothesized that an oscillation of tissue oxygen index (TOI) determined by near-infrared spectroscopy during recovery from exercise occurs due to feedback control of adenosine triphosphate and that frequency of the oscillation is affected by blood pH. In order to examine these hypotheses, we aimed 1) to determine whether there is an oscillation of TOI during recovery from exercise and 2) to determine the effect of blood pH on frequency of the oscillation of TOI. Three exercises were performed with exercise intensities of 30% and 70% peak oxygen uptake ($\dot{V}o_2peak$) for 12 min and with exercise intensity of 70% $\dot{V}o_2peak$ for 30 s. TOI during recovery from the exercise was analyzed by fast Fourier transform in order to obtain power spectra density (PSD). There was a significant difference in the frequency at which maximal PSD of TOI appeared (Fmax) between the exercises with 70% $\dot{V}o_2peak$ for 12 min (0.0039 ± 0 Hz) and for 30 s (0.0061 ± 0.0028 Hz). However, there was no significant difference in Fmax between the exercises with 30% (0.0043 ±0.0013 Hz) and with 70% $\dot{V}o_2peak$ for 12 min despite differences in blood pH and blood lactate from the warmed fingertips. It is concluded that there was an oscillation in TOI during recovery from the three exercises. It was not clearly shown that there was an effect of blood pH on Fmax.

Key words

Tissue oxygen index · Oscillation · Recovery from exercise · Power spectra density · Blood pH

Introduction

Richard (2003) simply explained the mechanism of oscillation in a dissipative structure by a model. The mechanism is represented by chemical equations and can be described as follows:

$G + A \longrightarrow 2A$ (1)

$A + B \longrightarrow 2B$ (2)

$B \longrightarrow$ (3)

This can be illustrated by translating it to an ecological system where the grass G is constantly supplied and animal A eats grass and reproduces as represented by Eq. 1. Animal B eats animal A and reproduces in Eq. 2. In Eq. 3 animal B is dying. This system exhibits limit cycle oscillations. The concentrations of A and B oscillate out of phase, i.e., at a high concentration of B, A is low, and a low concentration of A causes B to decrease. Subsequently, the low concentration of B allows A to increase, which is followed by an increase in B, and this continues with a regular pattern. A more detail introduction to oscillating chemical systems and their mathematical analysis is described, e.g., in Prigogine (1979).

In nonequilibrium thermodynamics, grass is energy supply for the system (input factor). The entropy produced in the system is eliminated from the system by death of B (output factor). In the system, free energy is available and consequently the system is self-organized. Such a system affected by input and output is called a dissipative structure.

In glycolysis in yeast, the input factor can be glucose. Continuous input of glucose can induce oscillation of NADH. Pulse input shows a damped oscillation with intervals of 37 sec (Chance *et al.* 1964, Richard 2003). Since within the system, B is a feedback factor, there should be a feedback factor of the stream of glycolysis. Phosphofructokinase (PFK) is an allosteric enzyme (Lodish et al. 2008). At high adenosine triphosphate (ATP) concentrations, PFK activity is low, resulting in a depletion of ATP synthesis. At high adenosine monophosphate (AMP) concentrations, PFK is activated, resulting in an increase in ATP. Thus, oscillations of chemical substances in glycolysis occur (DE La Fuente and Cortes 2012) and the process of chemical reactions is self-organized.

Phosphocreatine (PCr) kinetics during recovery from exercise has been examined in humans (Iotti et al. 2010). It has been shown that there is an oscillation of PCr during recovery, but oscillation of PCr during exercise and at rest has not been reported. During recovery, PCr is resynthesized by ATP produced by the hydrogen ion

difference between mitochondria membranes. In this study, an important factor was cytosolic pH. When cytosolic pH was low, oscillation frequency was low and vice versa. The electron transport chain consists of five complexes (Lodish *et al.* 2008). If oxygen is not consumed, it is thought that the electron transport chain (complexes I, III and IV) is stopped and consequently does not induce transportation of H^+ to the intermembrane space in mitochondria. Thus, oxygen consumption is thought to be essential for ATP production in complex V.

The recovery of PCr requires consumption of oxygen in skeletal muscle, suggesting oscillation in the tissue oxygen index (TOI), which can be determined by near-infrared spectroscopy (NIRS). According to Richard's model, the grass may be oxygen supply. Animal A may be regarded as oxygen consumption. A feedback signal may be derived from ATP (see Fig. 5). If this is the case, TOI could oscillate and the frequency may be affected by pH. Thus, the respiration system would be a complex dissipative structure (Iotti *et al.* 2010).

In the present study, we first examined whether there is an oscillation of TOI determined by NIRS during recovery from exercise. We secondly examined the effect of blood pH on frequency of the oscillation of TOI. The reason why we examined the oscillation of TOI during recovery is as follows. During recovery, consumption of ATP in skeletal muscle ceases and most of the lactate produced during exercise becomes an energy source. Therefore, the mechanism in the ATP supply system is much simpler during recovery than that during exercise. Another reason is due to the report in which the oscillation of PCr is examined only during recovery from the exercise (Iotti *et al.* 2010).

Methods

Subjects

Nine healthy males participated in this study. The means and standard deviations of ages, heights, body weights and peak oxygen uptake ($\dot{V}o_2peak$) levels of the subjects were 19.6 ± 1.4 yrs, 169.8 ±5.6 cm, 63.7 ± 8.7 kg and 3.18 ± 0.52 l/min, respectively. Each subject signed a statement of informed consent following a full explanation regarding the nature of the experiment. The Ethics Committee of Hokkaido University Graduate School of Education approved the present study. This study was performed in accordance with the Declaration of Helsinki.

Experimental protocol

Each subject performed three constant-load exercises and incremental ramp exercise until exhaustion on a cycle ergometer (Ergometer 232 CXL, Combi, Tokyo, Japan). After being in a resting state for 4 min, each subject performed constant-load exercise at 20 watts for 4 min, and then incremental ramp exercise was increased by 20 watts per one minute until the subject could not maintain the revolution rate of pedaling (60 rpm). $\dot{V}o_2$peak was determined by the maximal value during the incremental ramp exercise. In this determination, data of $\dot{V}o_2$ for 20 s were used. On another day, each subject performed three constant-load exercises: exercise with 30% of $\dot{V}o_2$peak determined by incremental ramp exercise for 12 min, exercise with 70% of $\dot{V}o_2$peak for 12 min and exercise with 70% of $\dot{V}o_2$peak for 30 s. Each subject rested for 10 min before the three exercises. The three exercises were followed by a recovery period of 20 min.

Before resting on the cycle ergometer seat prior to three constant-load exercises, each subject sat on a chair to attach electrodes on the subject's chest for monitoring heart rate (HR) and to attach photo probes on the subject's left leg (vastus lateralis) for NIRS. Each subject was instructed to relax and to maintain cycle ergometer cranking in a horizontal position at rest and during recovery on the cycle ergometer.

Measurements and determinations

Blood samples (each 100 µl) were collected from warmed fingertips using a capillary tube. Each subject's hand was pre-warmed in 40-45^{0}C water while sitting on the chair prior to each test in order to arterialize capillary blood (Zavorsky *et al.* 2007). After this warming, the subject's hand was warmed by a heating glove at rest, during exercise and during recovery on the cycle ergometer. It has been shown that such blood samples might not accurately reflect arterial O_2 pressure but can closely reflect arterial CO_2 and pH (21). Samples were analyzed using a blood gas analyzer (i-STAT1, i-STAT, Abbott Point of Care Inc. IL, USA) to measure CO_2 pressure ($PaCO_2$), pH and lactate (La).

Data for respiration gas exchange were obtained using a respiratory gas analyzer by the breath-by-breath mode (AEROMONITOR AE-310S, Minato Medical Science CO., LTD., Osaka, Japan). Ventilation ($\dot{V}E$) was measured by a hot-wire flow meter, and the flow meter was calibrated with a syringe of known volume (2 liters). O_2 and CO_2 concentrations were measured by a paramagnetic oxygen analyzer and photometric gas analyzer, respectively. The gas analyzer was calibrated by known standard gas (O_2: 15.13%, CO_2: 5.068%). Respiration gas exchange was measured

continuously during rest, exercise, and recovery periods. HR was recorded using a heart rate monitor installed in the respiratory gas analyser. $\dot{V}o_2$ and HR were obtained breath-by-breath. In incremental ramp exercise, breath-by-breath data were outputted as 20-s data.

TOI in the left vastus lateralis was determined using a NIRS system (NIRO200x, Hamamatsu Photonics, K. K. Hamamatsu, Japan). Although NIRO200x can determine oxygenation and deoxygenation by the Modified Beer-Lambert method, TOI determined by the spacially resolved spectroscopy (SRS) method was used in the present study. The NIRS probe consisted of a light source and an optical detector, with a distance of 3.0 cm between the light source and detector. Triple-wavelength light (735, 810 and 850 nm) emitted from the light source penetrates tissue, where it is either absorbed or scattered, and some of the scattered light returns to the optical detector. The sampling frequency of TOI was 1 Hz. TOI was calculated from deoxygenation (HHb) and oxygenation (O_2Hb) determined by the SRS method using the following equation:

$TOI = O_2Hb/(HHb + O_2Hb)$.

Calculation and statistical analysis

In a previous study, in order to obtain 1-s data, breath-by-breath data obtained in repeated exercise with a time interval were converted to 1-s data in each exercise, and the data obtained in each exercise were averaged (Whipp et al. 1982). However, in this method, the oscillation of measured data is eliminated by the averaging. In order to avoid this effect, breath-by-breath data were interpolated into 1-s data using a three-dimensional spine in the present study. However, there is also a problem in this method. Higher frequency of oscillation than respiration rate has no meaning.

The 1-s data for $\dot{V}o_2$ during recovery between 5 min and 20 min (We did not use data for the first 5 min during recovery because phase II appeared, especially in exercise with 70% $\dot{V}o_2$peak for 30 s: see results.) were analyzed by fast Fourier transform (FFT). TOI during recovery between 0 min to 20 min was analyzed by FFT. Power spectral density (PSD) was calculated with 4 windows. PSD for each frequency was individually normalized by the maximal peak value of PSD (PSDmax) by dividing PSD by PSDmax.

Results are presented as means ± standard deviations. Significant levels of peak frequency of PSD, blood pH and lactate among the three constant-load exercises were tested by the Tukey-HSP method if ANOVA showed significant levels. The significant level was set at $p<0.05$.

Results

Figure 1 shows typical examples of $\dot{V}o_2$ kinetics in the three exercises. $\dot{V}o_2$ increased rapidly (phase I) and then decreased during recovery from exercise for 30 s with 70% $\dot{V}o_2$peak. Thereafter, $\dot{V}o_2$ re-increased (phase II) and decreased during recovery. In the exercise with 30% $\dot{V}o_2$peak for 12 min, after a short phase I, $\dot{V}o_2$ showed phase II and then an uneven steady state (phase III). Then $\dot{V}o_2$ exponentially decreased. In the exercise with 70% $\dot{V}o_2$peak for 12 min, after a short phase I, $\dot{V}o_2$ exponentially increased (phase II) and then slowly increased (phase III). Thereafter, $\dot{V}o_2$ exponentially decreased. These phases of $\dot{V}o_2$ kinetics agree with previous reports (for example: Hughson et al. 1988, Özyener et al. 2001, Yano *et al.* 2014, Yano *et al.* 2007).

Tables 1 and 2 show blood lactate and pH levels at rest and during exercise and recovery. There were significant differences between the values in the exercise with 70% $\dot{V}o_2$peak and those in the exercise with 30% $\dot{V}o_2$peak or 70% $\dot{V}o_2$peak for 30 s.

Figure 2 shows averages of normalized PSDs of $\dot{V}o_2$ kinetics during recovery. Frequency at which PSDmax appeared (Fmax) of $\dot{V}o_2$ kinetics showed individually various values. There were also several peaks of PSD in each subject. Therefore, in order to smooth them, average PSDs are given in the figure. Fmax under the normalized PSDs were 0.0039 Hz in the exercise with 70% $\dot{V}o_2$ peak for 30 s, 0.0156 Hz in the exercise with 30% $\dot{V}o_2$peak for 12 min and 0.0156 Hz in the exercise with 70% $\dot{V}o_2$peak for 12 min.

Figure 3 shows the kinetics of TOIs in the three exercises. In the exercise with 70% $\dot{V}o_2$ peak for 30 s, TOI showed a rapid decrease and then recovered to the resting level. TOI sometimes overshot the resting level during recovery and showed oscillation. In the exercise with 30% $\dot{V}o_2$peak for 12 min, TOI showed a slight decrease or steady state and then recovered to resting level. There was oscillation of TOI. In the exercise with 70% $\dot{V}o_2$ peak for 12 min, TOI decreased during exercise and recovered to the resting level during recovery. There was also oscillation of TOI during recovery in this exercise. It seemed that there were also oscillations of TOI at rest and during exercise in the three exercises, although these oscillations at rest and during exercise were not analyzed further in this study.

Figure 4 shows PSDs of TOI in the three exercises. There were individually various values in Fmax of TOI in the exercise with 70% $\dot{V}o_2$peak for 30 s. The average ± SD of frequency was 0.0061 ± 0.0028 Hz. In the exercise with 30% $\dot{V}o_2$peak for 12

min, one subject showed three peaks. When the maximal peak was eliminated, there was no difference in Fmax (0.0039 Hz). When the exception was included, the average ± SD of frequency was 0.0043 ± 0.0013 Hz. There was no difference in Fmax of TOI in the exercise with 70% $\dot{V}o_2$peak for 12 min. The average of frequency was 0.0039 Hz. There was a significant difference in Fmax between the exercises with 70% $\dot{V}o_2$peak for 30s and that for 12 min. However, there was no significant difference in Fmax between the exercise with 30% $\dot{V}o_2$peak for 12 min and that with 70% $\dot{V}o_2$peak for 12 min despite the significant differences in blood lactate and blood pH levels between them.

Discussion

Figure 5 shows the energetics of exercise not only during exercise but also during recovery from the exercise. At the onset of exercise, $\dot{V}o_2$ increased exponentially, indicating a lack of energy (oxygen deficit). This oxygen deficit consists mainly of the energy source from PCr in exercise of low intensity and the energy source from glycolysis is added in the case of high intensity of exercise. This results in an increase in blood lactate. During recovery from the exercise, CPr can be recovered by oxidative phosphorylation. Eighty percent of the increased lactate is used for oxygen consumption during recovery as an energy source (Brooks 2000). Activation of glycolysis during exercise is thought to almost cease during recovery. Thus, energy sources during recovery would be mainly derived from the oxidative phosphorylation process.

An oxygen delivery and metabolic control hypothesis has been proposed. In this hypothesis, a key concept is that a given ATP production can be attained across a range of intracellular oxygen pressures (Po_2) by alternating the concentrations of other substrates (Hughson *et al.* 2001). In fact, it has been reported that intramuscular Po_2 becomes 3 to 5 mmHg across a wide range of exercise intensities (Richardson *et al.* 1995) and that muscle PCr depletion is affected by hypoxia and hyperoxia (Haseler *et al.* 1998). Accordingly, these findings suggest that intramuscular Po_2 is low during exercise and probably during recovery and that at low intramuscular Po_2, PCr works to compensate the lack of intramuscular Po_2 operation for oxidative phosphorylation. Phosphate (Pi) and Ca^{2+} in relation to muscle contraction are also regarded as the factors affecting oxidative phosphorylation (Tschakovsky and Hughson 1999, Schmitz *et al.* 2012). Thus, the homeostasis of ATP is maintained (Balaban, 2009) (Since we postulate that there is oscillation of ATP during exercise and recovery, we should call it homeodynamics rather than homeostasis.).

$\dot{V}o_2$ oscillated during recovery, but there were several peaks in frequency of $\dot{V}o_2$.

$\dot{V}o_2$ is determined at the lung level, and $\dot{V}o_2$ oscillation is therefore affected not only by oscillation of cardiorespiration (Yano et al. 2014) but also by oscillation of mixed venous oxygen content, which should be affected by TOI. Therefore, PSD distribution on $\dot{V}o_2$ would be complex rather than that on TOI.

It has been reported that PCr re-synthesis can oscillate during recovery in humans. The frequency ranges from 0.002 to 0.025 Hz (Iotti *et al.* 2010). The frequency at which maximal peak of TOI as well as $\dot{V}o_2$ appeared during recovery in the present study is within this range. The PCr oscillation suggests oscillations of Pi and ATP. If there are feedback loops in such substrates and homeodynamics of ATP is also postulated during recovery, TOI could oscillate during recovery. In fact, deoxygenation determined by the Beer-Lambert method (BLM) was observed to oscillate, although this observation was made during exercise (Yano *et al.* 2014a, Yano *et al.* 2013b).

We used TOI determined by spatially resolved spectroscopy (SRS) instead of deoxygenation determined by BLM. The estimation of deoxygenation by BLM in previous studies has been reported to be affected by skin blood flow under a certain condition (Buono *et al.* 2005, Davis *et al.* 2005), though the validation of this method has been reported (Mancini *et al.* 1994, Tran *et al.* 1999). Furthermore, skin blood flow is known to oscillate at several bands in frequency (0.009 – 1.6 Hz) including low frequency band (0.009 - 0.02 Hz) (Kvernmo *et al.* 1998, Kvernmo *et al.* 1999). This suggests that skin blood flow affects the frequency of deoxygenation. However, it has been shown that estimation of TOI by SRS is not greatly affected by oxygenation dynamics of skin blood flow (Messere and Roatta, 2013).

It has been hypothesized that cytosolic pH affects frequency of oscillation of PCr re-synthesis during recovery. It has been confirmed that Fmax of deoxygenation at rest decreased in exercise, but there was no result for blood pH and deoxygenation was determined by BLM in the previous study (Yano *et al.* 2013). There were contradictory results although blood pH was measured and SRS was used in the present study. Accordingly there was a difference in the Fmax between exercises with 70% $\dot{V}o_2$peak for 30s and 12 min. However, there was no difference in the Fmax between exercises with 30% $\dot{V}o_2$peak and with 70% $\dot{V}o_2$peak for 12 min. There might be another factor which becomes the low Fmax like the present exercise with 30% $\dot{V}o_2$peak. Furthermore, being not equivalent to cytosolic pH, blood pH gradually recovered to the resting level during recovery. It is difficult for this time variation to be evaluated by the FFT method. This is a limitation of the present study. In any case, further examination is needed.

In conclusion, there was oscillation in TOI during recovery from exercise. It

seems that the oscillation occurs at rest and during exercise. The results suggested that there are feedback loops from ATP as well as Pi to the oxidative phosphorylation process. This may cause the oscillation of TOI. It was not clearly shown that blood pH had an effect on Fmax of TOI. This is probably due to usage of blood pH. Cytosolic pH could affect the shift of Fmax of TOI but since blood pH does not always reflect cytosolic pH, the effect of cytosolic pH on Fmax of TOI could not clearly be elucidated by the measurement of blood pH.

Conflict of Interest

There is no conflict of interest.

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Table 1. Mean values and standard deviation (SD) of arterialized blood lactate at rest, during exercise (Ex) and during recovery (Rec).

		Rest	Ex 5min	Ex 10min	Rec 5min	Rec 10 min	Rec 20 min
30%-12min	mean	1.32	1.58	1.38	1.10	1.01	1.00
	SD	0.39	0.38	0.37	0.26	0.27	0.29
70%-30 sec	mean	1.17			2.00	1.65	1.33
	SD	0.42			0.51	0.38	0.26
70%-12min	mean	1.01	8.10*	10.21*	8.97*#	7.40*#	4.76*#
	SD	0.21	1.89	3.10	3.98	3.95	2.92

* significant difference compared to the exercise with 30% $\dot{V}o_2$peak for 12 min (30%-12min). # significant difference compared to the exercise with 70% $\dot{V}o_2$peak for 30 s (70%-30sec). 70% $\dot{V}o_2$peak for 12 min was expressed as 70%-12 min.

Table 2. Mean values and standard deviation (SD) of arterialized blood pH at rest, during exercise (Ex) and during recovery (Rec).

		Rest	Ex 5min	Ex 10min	Rec 5min	Rec 10min	Rec 20min
30%-12min	mean	7.39	7.36	7.37	7.39	7.39	7.38
	SD	0.02	0.02	0.03	0.02	0.02	0.02
70%-30 sec	mean	7.39			7.36	7.38	7.38
	SD	0.01			0.02	0.03	0.02
70%-12min	mean	7.39	7.27*#	7.28*#	7.28*#	7.30*#	7.36*#
	SD	0.02	0.05	0.07	0.07	0.06	0.04

* significant difference compared to the exercise with 30% $\dot{V}o_2$peak for 12 min (30%-12min). # significant difference compared to the exercise with 70% $\dot{V}o_2$peak for 30 s (70%-30sec). 70% $\dot{V}o_2$peak for 12 min was expressed as 70%-12 min.

Legends of figures

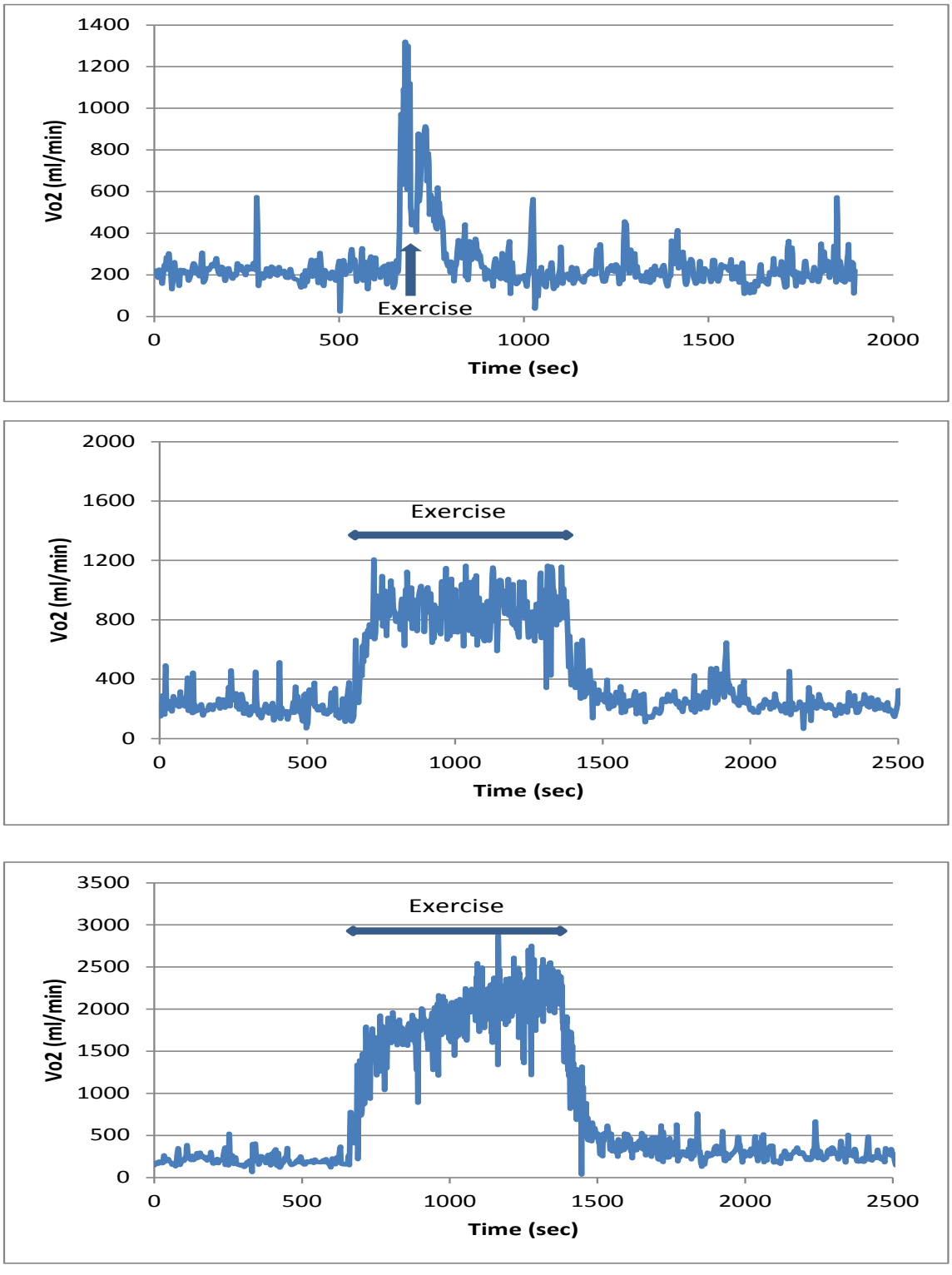
Fig. 1. Oxygen uptake ($\dot{V}o_2$) kinetics in the three exercises. The upper panel shows $\dot{V}o_2$ kinetics of the exercise with 70% $\dot{V}o_2$peak for 30 s. The middle shows $\dot{V}o_2$ kinetics of the exercise with 30% $\dot{V}o_2$peak for 12 min. The lower shows $\dot{V}o_2$ kinetics of the exercise with 70% $\dot{V}o_2$peak for 12 min. Arrows show the period in which was performed exercise.

Fig. 2. Average power spectra density (PSD) for oxygen uptake obtained during recovery from the exercise with 70% $\dot{V}o_2$peak for 30 s (upper panel), the exercise with 30% $\dot{V}o_2$peak for 12 min (middle panel) and the exercise with 70% $\dot{V}o_2$peak for 12 min (lower panel).

Fig. 3. Tissue oxygen index (TOI) kinetics in the three exercises. The upper panel shows TOI kinetics with the exercise of 70% $\dot{V}o_2$peak for 30 s, the middle shows TOI kinetics of the exercise with 30% $\dot{V}o_2$peak for 12 min, and the lower shows TOI kinetics of the exercise with 70% $\dot{V}o_2$peak for 12 min. Arrows show the period in which was performed exercise.

Fig. 4. Power spectra density (PSD) for tissue oxygen index individually obtained during recovery from the exercise with 70% $\dot{V}o_2$peak for 30s (upper panel), the exercise with 30% $\dot{V}o_2$peak for 12 min (middle panel) and the exercise with 70% $\dot{V}o_2$peak for 12 min (lower panel).

Fig. 5. A simplified block diagram indicating energetics in the skeletal muscle system and input and output of substances in the system. The input of free fatty acid and the output of heat are not illustrated. The dotted line represents the energetic process during muscle contraction. The solid line represents the energetic process during recovery from muscle contraction.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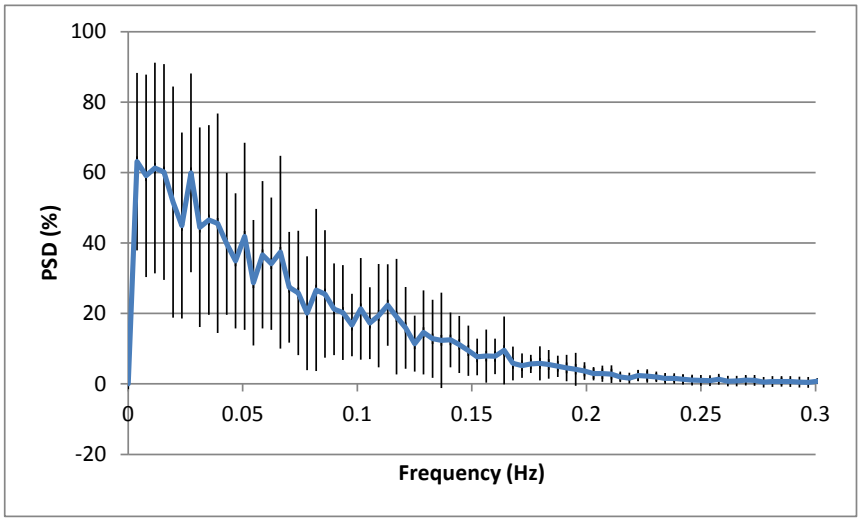
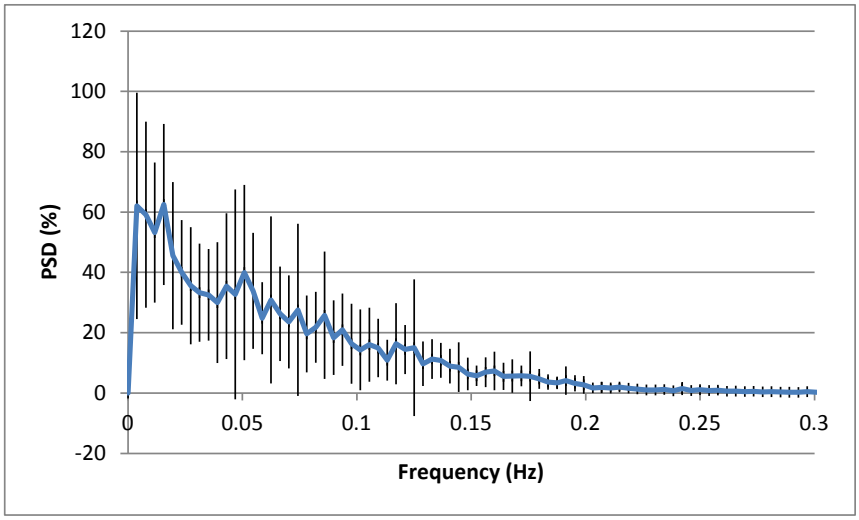
Time (sec)

Fig. 1.


100
80
60
40
20
0
-20
PSD (%)
0
0.05
0.1
0.15
0.2
0.25
0.3
Frequency (Hz)


120
100
80
60
40
20
0
-20
PSD (%)
0
0.05
0.1
0.15
0.2
0.25
0.3
Frequency (Hz)

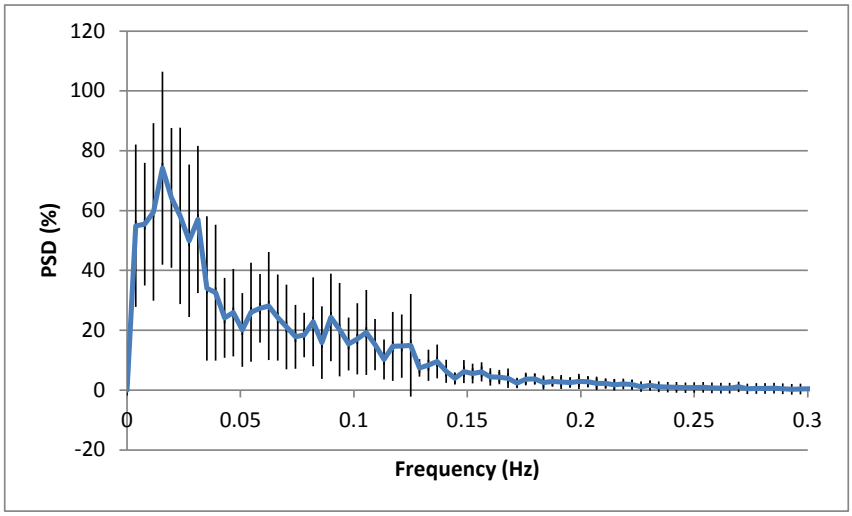
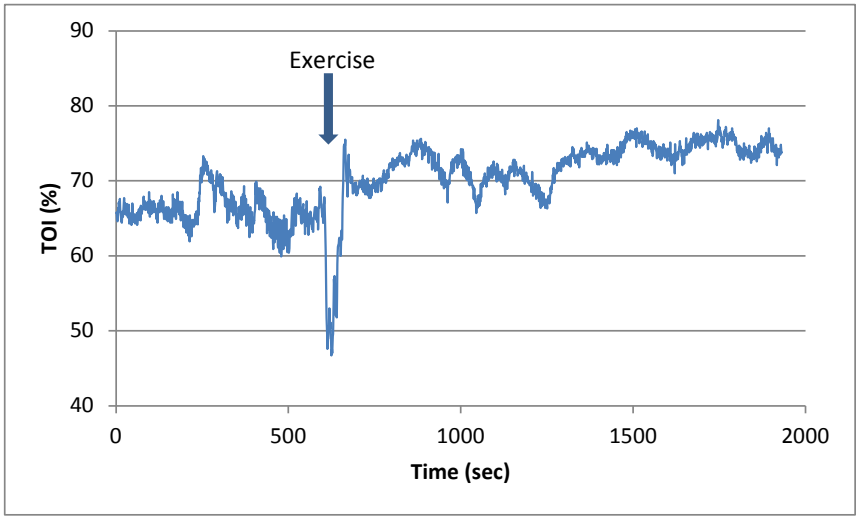
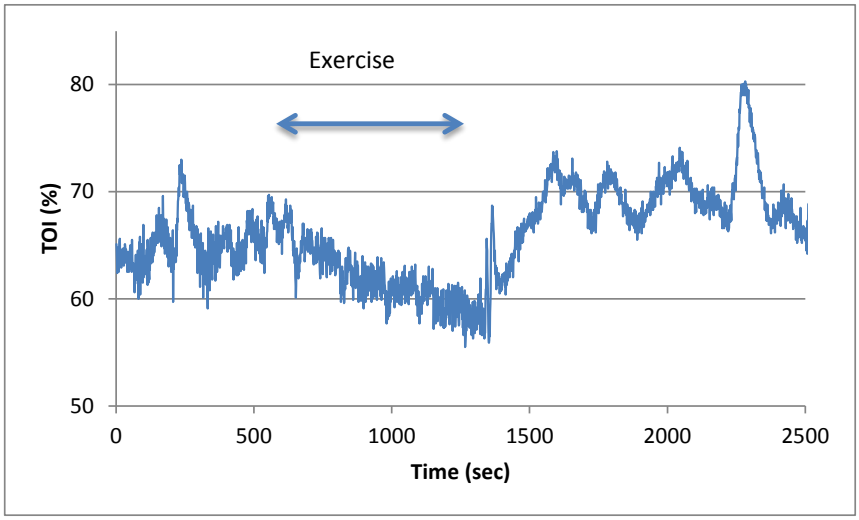

120
100
80
60
40
20
0
-20
PSD (%)
0
0.05
0.1
0.15
0.2
0.25
0.3
Frequency (Hz)

Fig. 2.


Exercise
TOI (%)
90
80
70
60
50
40
0
500
1000
1500
2000
Time (sec)


Exercise
TOI (%)
80
70
60
50
0
500
1000
1500
2000
2500
Time (sec)

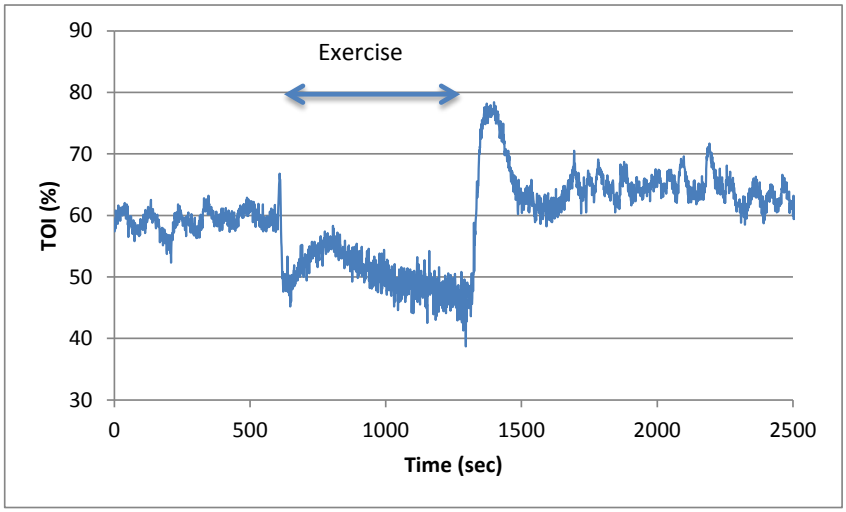
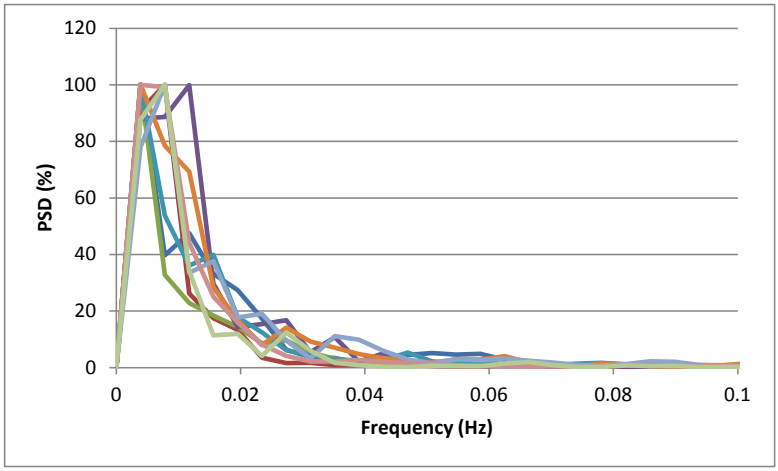
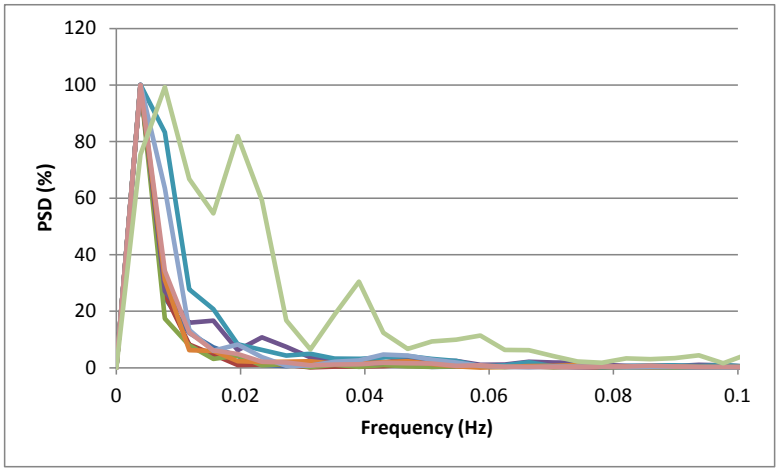

Exercise
TOI (%)
90
80
70
60
50
40
30
0
500
1000
1500
2000
2500
Time (sec)

Fig. 3


120
100
80
60
40
20
0
PSD (%)
0
0.02
0.04
0.06
0.08
0.1
Frequency (Hz)


120
100
80
60
40
20
0
PSD (%)
0
0.02
0.04
0.06
0.08
0.1
Frequency (Hz)

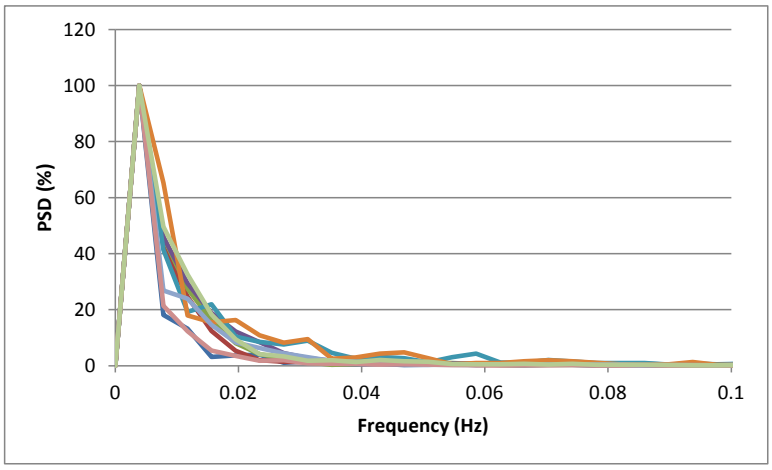

120
100
80
60
40
20
0
PSD (%)
0
0.02
0.04
0.06
0.08
0.1
Frequency (Hz)

Fig. 4

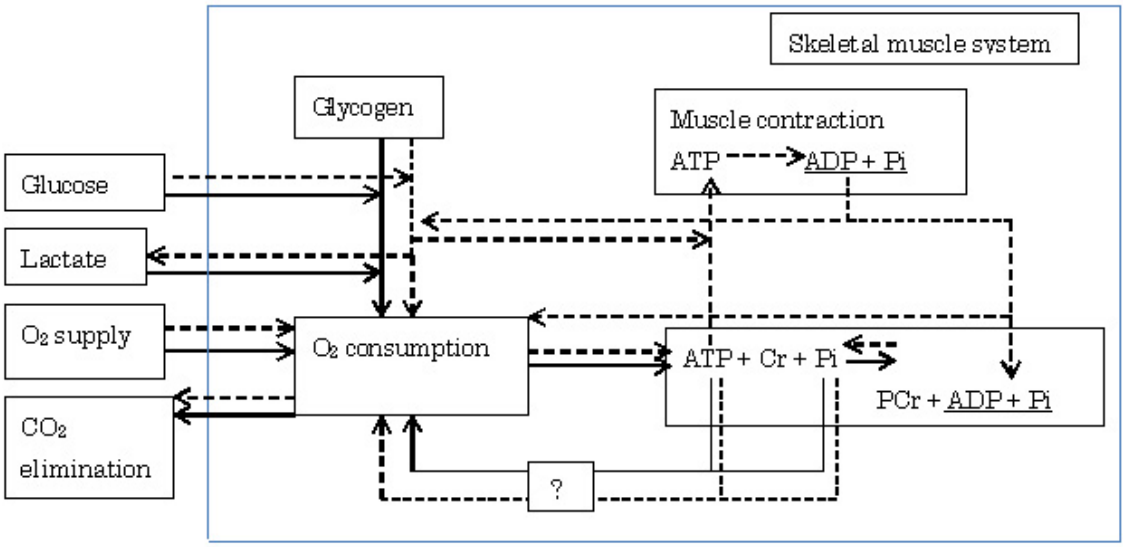

Skeletal muscle system
Glycogen
Muscle contraction
ATP
ADP + Pi
Glucose
Lactate
O2 supply
O2 consumption
ATP + Cr + Pi
PCr + ADP + Pi
CO2
elimination
?

Fig. 5.