Difference between low and high intensity exercises in the amplitude of oscillation of tissue oxygen index.

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Key words

Tissue oxygen index - Amplitude of oscillation - Exercise intensity - Power spectral density

Abstract

Aim. We hypothesized that an increase in the interaction of the biochemical substance due to the high exercise intensity could increase the amplitude of oscillation in TOI. In order to test this hypothesis, we examined whether oscillation of the tissue oxygen index (TOI) during exercise was influenced by the difference between low and high intensity exercises.

Methods. Nine subjects performed low-intensity exercise (Low-E) and high-intensity exercise (High-E) for 12 min. TOI was measured from the vastus lateralis muscle by near-infrared spectroscopy. TOI from 3 min to 12 min during exercise was analyzed by fast Fourier analysis to obtain power spectral density (PSD). PSD was integrated in the low frequency range (<0.025 Hz).

Results. The maximal peak of PSD appeared at 0.0039 ± 0 Hz in Low-E and at 0.0048 ± 0.002 Hz in High-E. There was no significant difference in the maximal peak of PSD between the two exercises. PSD at 0.016 Hz during High-E (43 ± 33 $\%^2$/Hz) was significantly higher than that during Low-E (13 ±6 $\%^2$/Hz). The integrated PSD was significantly larger in High-E (2.0 ±1.6 $\%^2$) than in Low-E (0.9 ± 0.6 $\%^2$).

Conclusion. The results suggest that interactions among biochemical substances involved in oxidative metabolism occur more strongly in High-E than in Low-E.

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INTRODUCTION

If each biochemical substance in the TCA cycle is scattered in mitochondria, each chemical reaction should occur randomly. Self-organization is necessary for the reactions to occur orderly. The important theory is dissipative structure [1]. This is the theory that each biochemical substance interacts to create an overall order. However, it is not easy to obtain evidence that a chemical reaction in a living body is a dissipative structure. One piece of evidence is oscillation of the concentration of a biochemical substance that appears as a result of each biochemical substance interacting. In yeast, oscillation of NADH in the process of glycolysis has been confirmed [2], and its theoretical simulation has been performed [3].

Iotti et al. [4] first suggested that this dissipative structure exists in energy metabolism in humans. Their suggestion was based on the fact that the recovery process of creatine phosphate (CrP) oscillated after exercise. The frequency of the oscillation was very slow (0.002 - 0.025 Hz). This frequency was inversely proportional to cytosolic pH. From these results, Iotti et al. [5] suggested that the oscillation during recovery is derived from the interaction of several biochemical processes involving oxidative phosphorylation and several transport systems across mitochondrial and plasma membranes and their regulation and that cytosolic pH has a pivotal role in the pattern of CrP recovery.

It has been reported that the oxygenation dynamics of skeletal muscle determined by near-infrared spectroscopy (NIRS) using the Beer-Lambert method oscillates at rest and during exercise [6, 7]. The oxygenation kinetics obtained by this method is affected by skin blood flow [8]. It has been reported that oscillations of skin blood flow and oxygenation kinetics do not necessarily match each other and that oscillation of skin blood flow includes a low frequency band [9]. Therefore, since the tissue oxygenation index (TOI) determined by spatially resolved spectroscopy (SRS) is hardly affected by skin blood flow [8], it has been confirmed again that TOI oscillates in inactive muscle during exercise [10] and during recovery from exercise [11]. Furthermore, they reported that peak power spectral density in TOI (0.0039 – 0.0078 Hz) was within the very low frequency range reported by Iotti et al. [4, 5] and they suggested that the oscillation of TOI is derived from muscle oxidative metabolism [10, 11].

Richard [2] proposed a simple model of oscillation of the concentrations of biochemical substances. Oscillation of TOI is interpreted with reference to this model as follows [10, 11]. Oxygen starts to increase as exercise begins. Oxygen consumption increases accordingly. As a result, ATP is produced. If ATP is not sufficiently consumed by muscle contraction, ATP increases. It decreases when ATP suppresses oxygen consumption by feedback action. This suppression reduces oxygen consumption. As a result, ATP decreases. Oxygen consumption and ATP oscillate in this process. In fact, ATP seems to oscillate at a frequency of 0.023 Hz (personal communication from co-author, Arimitsu).

Thus, the oscillation of TOI could be produced by the interactions of biochemical substances in oxidative metabolism. However, since TOI oscillation is macroscopic

phenomena, it must be assumed that the microscopic oscillation of oxygen consumption exists and is synchronized among mitochondria. Since it is difficult to obtain such microscopic evidences in humans, it is necessary to obtain further macroscopic evidences. As a further step, we hypothesized in the present study that an increase in the interaction of the biochemical substance due to the high exercise intensity could increase the amplitude of oscillation in TOI. In order to test this hypothesis, we examined whether the amplitude of oscillation of TOI during exercise is influenced by the difference between low and high intensity exercises.

Materials and methods

Subjects

Nine healthy males participated in this study. The means and standard deviations of ages, heights, body weights and peak oxygen uptake ($\dot{V}o_2$peak) levels of the subjects were 19.6 ± 1.4 years, 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 two 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 W for 4 min, and then incremental ramp exercise was increased by 20 W per one min 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 two constant-load exercises: exercise with 30% of $\dot{V}o_2$peak (Low-E) determined by incremental ramp exercise for 12 min and exercise with 70% of $\dot{V}o_2$peak for 12 min (High-E). Each subject rested for 10 min before the two exercises. The two exercises were followed by a recovery period of 20 min. Before resting on the cycle ergometer seat prior to the two 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 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

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 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 analyzer. $\dot{V}o_2$ and HR were obtained breath-by-breath. In incremental ramp exercise, breath-by-breath data were outputted as 20-s data.

TOI in the vastus lateralis was determined using a near-infrared spectroscopy (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 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 SRS using the following equation:

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

Blood samples (each 100 μl) were collected from warmed fingertips using a capillary tube. Each subject's hand was pre-warmed in 40-45 °C water while sitting on the chair prior to each test in order to arterialize capillary blood [12]. After this warming, the subject's hand was warmed by a heating glove at rest and during exercise 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 pH [12]. Samples were analyzed using a blood gas analyzer (i-STAT1, i-STAT, Abbott Point of Care Inc. IL, USA) to measure pH.

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 [13]. However, in this method, oscillation of the data obtained is eliminated by the averaging. A way to obtain second-by-second data from breath-by-breath data was developed in a recent study [14] but average data for four trials were also used. Therefore, in final data obtained by these treatments, $\dot{V}o_2$ oscillation, which should be affected by HR variability [15], is finally cancelled. In order to avoid this effect, breath-by-breath and HR data were interpolated into 1-s data using a three-dimensional spine curve for one trial data for $\dot{V}o_2$ 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 TOI during exercise between 3 min and 12 min (Data for the first 3

min of exercise were not used because of a rapid increase.) were analyzed by fast Fourier transform (FFT). Power spectral density (PSD) was calculated with 2 windows. PSD was numerically integrated in the very low frequency range (very low frequency component <0.025 Hz). Data at rest for 10 min, during exercise for 12 min and during recovery for 20 min that were processed by low-pass filtering (<0.05 Hz). Due to the filtering with treatment of removal of trend, TOI, HR and $\dot{V}O_2$ become the values changed from average in whole data, respectively (Fig. 2).

Results are presented as means ± standard deviations. Significant differences in PSD, very low frequency component, frequency at maximal PSD and blood pH between the two constant-load exercises were tested by the paired t-test. Significant differences from resting values for blood pH were tested by Dunnett's method. The significant level was set at $p<0.05$.

Results

Figure 1 shows typical examples of TOI, HR and $\dot{V}O_2$ at rest, during exercise and during recovery. There was a significant difference between TOI at 12 min during Low-E (68 ± 4.2%) and that during High-E (56 ± 5.9%). There was a significant difference between HR at 12 min during Low-E (116 ± 8.2 beats /min) and that at 12 min during High-E (180 ± 4.9 beats/min). There was a significant difference between $\dot{V}O_2$ for the last minute of Low-E (1.24 ±0.17 l/min) and that for the last minute of High-E (2.77 ± 0.18 l/min). There was a continuous increase after a rapid increase (slow component) in High-E. The slow component leads to an increase in exercise intensity in High-E [16].

Figure 2 shows typical examples of TOI, HR and $\dot{V}O_2$ after processing the data shown in Figure 1 by low-pass filtering (<0.05 Hz). These values appeared to have very low frequency waves.

Figure 3 shows the individual PSD for TOI during Low-E and High-E (panels A and B). Panel C shows average values. Maximal peaks were at 0.0039 ± 0 Hz in Low-E and 0.0048 ± 0.002 Hz in High-E. There was no significant difference in the frequency between them. The PSD that was close to the maximal peak and showed a significant difference between the two exercises appeared at 0.016 Hz (This frequency was within the range in oscillation of creatine phosphate re-synthesis reported by Iotti et al. [5].). That is, PSD at 0.016 Hz was significantly higher in High-E (43 ± 33 $\%^2$/Hz) than in Low-E (13 ± 6 $\%^2$/Hz) (panel A in Figure 4). In this frequency, PSD of one subject was lower in High-E than in Low-E. However, the difference was very small. In the other subjects, PSDs showed larger values in High-E than in Low-E. The very low frequency component obtained below 0.025 Hz was significantly larger in High-E (2.0 ± 1.6 $\%^2$) than in Low-E (0.9 ± 0.6 $\%^2$) (panel B in Figure 4).

Figure 5 shows changes in blood pH in each exercise. In High-E, blood pH was significantly different from the levels at rest, but there were no significant differences in Low-E. Blood pH in High-E were significantly lower than those in Low-E during exercise for 12 minutes.

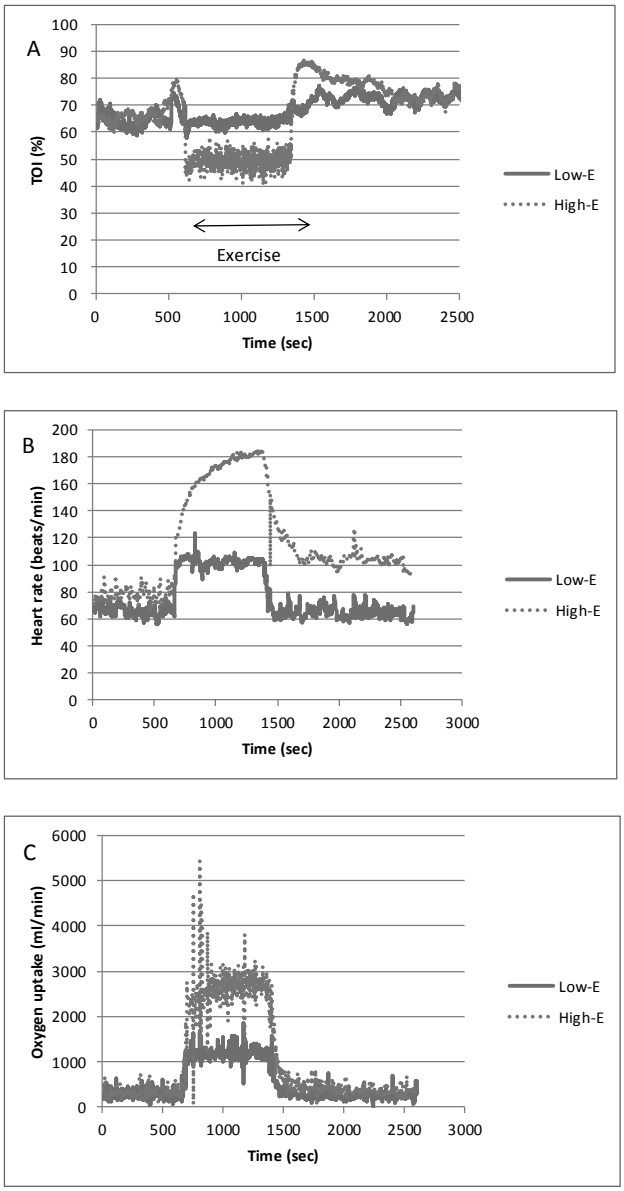

Figure 1

Typical examples of tissue oxygen index (TOI) (Panel A), heart rate (Panel B) and oxygen uptake (Panel C). Data at rest for 10 min, during exercise for 12 min and during recovery for 20 min are shown. Two exercises, low-intensity exercise (Low-E) and high-intensity exercise (High-E), were carried out for 12 min.

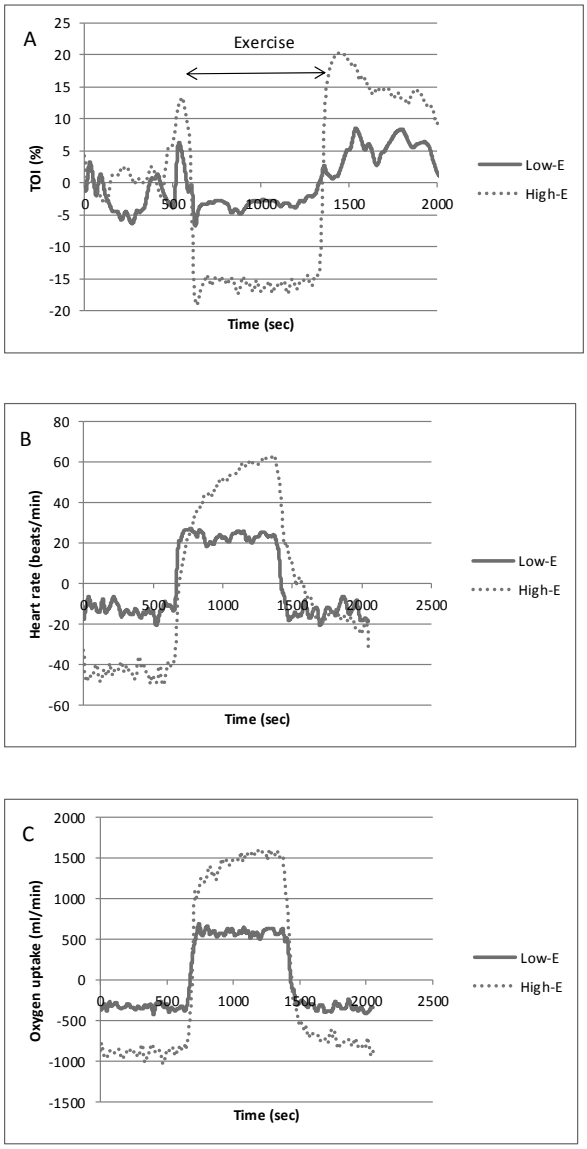

Figure 2

Typical examples of tissue oxygen index (TOI) (A), heart rate (B) and oxygen uptake (C). Data at rest for 10 min, during exercise for 12 min and during recovery for 20 min that were processed by low-pass filtering (<0.05 Hz) are shown. Two exercises, low-intensity exercise (Low-E) and high-intensity exercise (High-E) were carried out. Due to the filtering with treatment for removal of trend, TOI, HR and oxygen uptake became values that were different from averages in whole data.

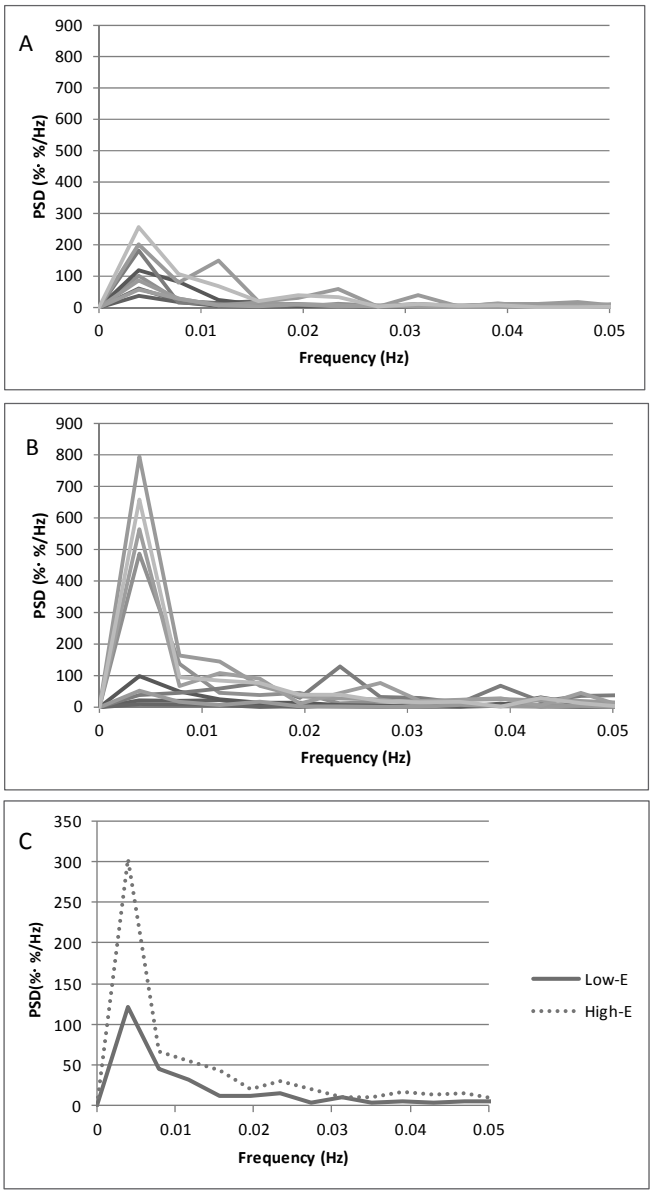

Figure 3

Power spectra density (PSD) for tissue oxygen index (TOI) individually obtained during exercise from 3 min to 12 min in low-intensity exercise (Panel A) and in high-intensity exercise (Panel B). Averaged power spectra density (PSD) for TOI obtained during low-intensity exercise (Low-E) and high-intensity exercise (High-E) (Panel C).

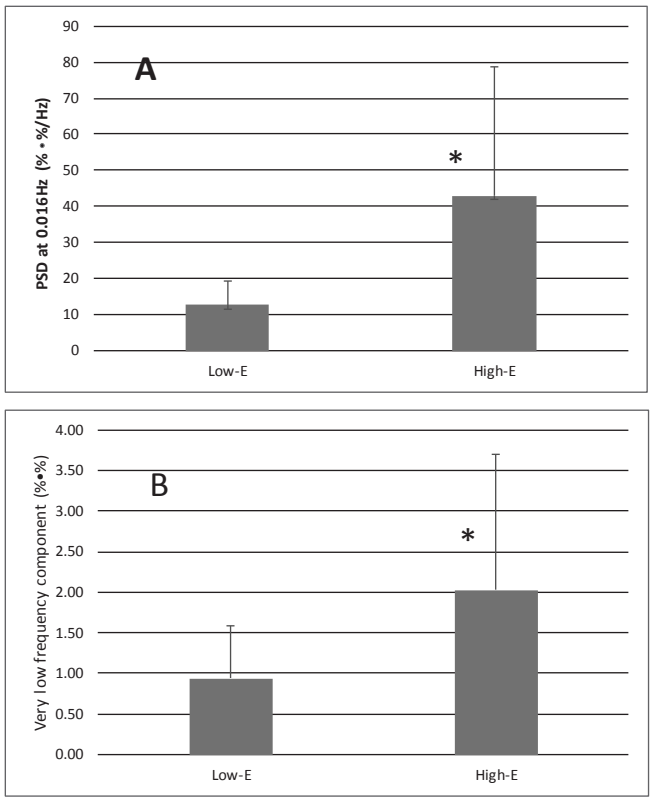

Figure 4

Comparison between very low frequency components or power spectra densities (PSDs) at 0.016 Hz obtained in the low-intensity exercise (Low-E) and high-intensity exercise (High-E). Panel A shows PSD at 0.016 Hz. Panel B shows the very low frequency component obtained below 0.025 Hz. *: significant difference between the exercises. The low frequency component was obtained by numerical integration of power spectral density.

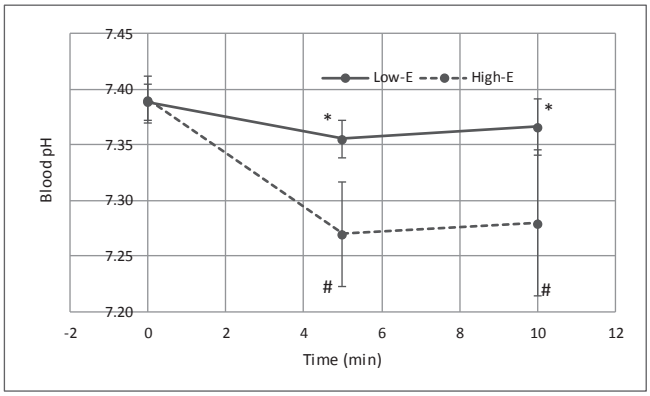

Figure 5

Mean values and standard deviation (SD) of arterialized blood pH at rest (time=0 minute), and during exercise. *: siginificant difference between low-intensity exercise (Low-E) and high-intensity exercise (High-E). #: significnat difference compared to the level at rest.

Discussion

Oxygen uptake of lungs and oxygen consumption of muscles: Oxygen uptake determined in the lungs had been assumed to increase exponentially, but it has been proven to oscillate under the influence of heart rate oscillation [15]. However, this does not suggest oscillation of oxygen consumption in working muscles. If the oxidative metabolism of working muscles is a dissipative structure, the concentration of the biochemical substance related to the process would oscillate.

TOI synchronization: It has been hypothesized that reactive oxygen species (ROS) are trigger factors that synchronize mitochondrial activity in cardiac muscle of the Guinea pig on the basis of the following results [17]. NADH generated from the TCA cycle is transported to complex I of the electron transport system. Oxygen is used in complex IV, but a part of it forms ROS in complex III. In this process, the mitochondrial inner membrane potential, NADH and ROS oscillate. Complexes I, III, and IV transfer hydrogen ions from the inner space of mitochondria to the intermembrane space. In complex V, ATP is produced as hydrogen ions migrate into the inner space. ROS go out of the mitochondria and activate other mitochondria. That is, the oscillation of ROS generated in many mitochondria may be a synchronization factor among mitochondria. However, ROS was not measured in the present study because in humans there is no way to measure ROS non-invasively and continuously.

Relationship between amplitude and PSD: The unit of amplitude of TOI is %. In PSD it is $\%^2$ / Hz. From the relationship of units, amplitude magnitude is expanded in PSD. For example, if the amplitude is 1% (see the oscillation of TOI during exercise in Figure 2) when the frequency is 0.01 Hz, PSD is $100\%^2$/ Hz (1^2/0.01). It is not known whether there is a device physiologically increasing the actual TOI amplitude to the PSD value in the muscular and nervous system. However, since this relationship is simple, it is considered that the PSD value represents TOI amplitude by frequency.

Increase of PSD due to exercise intensity: Many chemical reactions occur in the TCA cycle. The reactions lead to interactions among biochemical substances. These interactions can cause oscillation. In fact, NADH generated in the reactions oscillates [18]. Since NADH is transferred to the electron transfer system, there is a possibility that all of the electrons in the electron transfer system oscillate. If this is the case, the concentration of oxygen consumed by complex IV would oscillate. ATP produced by complex V should also oscillate. Furthermore, there can be feedback from ATP to oxygen concentration as mentioned in the Introduction [10, 11]. If ATP oscillates, oxygen consumption also would oscillate.

When exercise intensity is increased, the TCA cycle is more strongly activated, inducing enhancement of the interactions among the biochemical substances. As a result, the interactions would increase not only the level of NADH but also the amplitude of NADH oscillation. The increase in NADH affects oxygen consumption through the electron transport system. At the same time, there would be propagation of the increased

oscillation in NADH to oxygen consumption. Also, due to increased consumption and production of ATP, the oscillation of ATP should be enhanced. This enhancement can induce an increase in the amplitude of oscillation in oxygen concentration because oxygen consumption should be regulated to maintain homeodynamics of ATP by feedback from ATP to oxygen consumption. Furthermore, these microscopic phenomena can expand to macroscopic phenomena through synchronization among mitochondria. Thus, activation by the microscopic interactions among biochemical substances in the mitochondrion due to an increase of exercise intensity can appear as an increase in the macroscopic amplitude of oscillation of TOI through synchronization.

Effect of blood pH on frequency: It has been reported that the recovery process of CrP after local muscle exercise oscillates, and this frequency is inversely proportional to cytosolic pH. That is, when exercise intensity is low, cytosolic pH is high and the frequency of re-synthesis of CrP is slow [4, 5]. Cytosolic pH has a pivotal role in the pattern of CrP recovery due to oxidative phosphorylation [5]. In our previous study, we compared the frequency of maximal peak value in each PSD of TOI during recovery from two types of exercise showing normal blood pH and from one type of exercise showing low blood pH, but a definite tendency was not found 11. In the present study, a comparison was made during exercise between the two exercise intensities, but there was no difference in the frequencies of the maximal peak values in PSD of TOI. These are results for blood pH, which is different from the cytosolic pH value, but if exercise intensity is changed, cytosolic pH should also be very different especially during exercise. Thus, in the present study, it could not be clear whether hydrogen ions particularly act in interactions of biochemical substances in relation to oxidative metabolism, although there is a possibility that hydrogen ions may affect the amplitude of oscillation of TOI.

Conclusions

The results of this study suggest that interactions among biochemical substances involved in oxidative metabolism occur more strongly in high-intensity exercise than in low-intensity exercise. The results provide further evidence that TOI oscillation is derived from the interactions among substances related to muscle oxidative metabolism. It could not be clear whether hydrogen ions particularly act in interactions of biochemical substances in relation to oxidative metabolism.

Conflict of interest: We declare no conflicts of interest.

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低強度および高強度運動時の組織酸素指数の振動の差異

矢野徳郎・アフロンド ロガイエ・有光琢磨・柚木孝敬

【要旨】 高い運動強度による生化学物質の相互作用の増加は、TOIにおける振動の振幅を増加させる可能性があると仮説した。この仮説を検証するために、我々は、運動中の組織酸素指数（TOI）の振動が、低強度運動と高強度運動の違いによって影響を受けるかどうかを調べた。低強度運動（Low-E）および高強度運動（High-E）を12分間行った。TOIは、近赤外分光法により外側広筋から測定した。運動中の3分から12分までのTOIを、パワースペクトル密度（PSD）を得るための高速フーリエ解析によって分析した。低周波帯で積分されたPSDは、High-E（2.0±1.6%2）ではLow-E（0.9±0.6%2）よりも有意に大きかった。これらの結果は、酸化的代謝に関与する生化学物質間の相互作用が、Low-EよりもHigh-Eにおいてより強く生じることを示唆している。

【キーワード】組織酸素指数，振動の振幅，運動強度，パワースペクトル密度