

COMPARISON OF OSCILLATIONS OF SKIN BLOOD FLOW AND DEOXYGENATION IN VASTUS LATERALIS IN LIGHT EXERCISE

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ABSTRACT: The purpose of the present study was to compare oscillation of skin blood flow with that of deoxygenation in muscle during light exercise in order to determine the physiological significance of oscillations in deoxygenation. Prolonged exercise with 50% of peak oxygen uptake was performed for 60 min. Skin blood flow (SBF) was measured using a laser blood flow meter on the right vastus lateralis muscle. Deoxygenated haemoglobin/myoglobin (DHb/Mb) concentration in the left vastus lateralis were measured using a near-infrared spectroscopy system. SBF and DHb/Mb during exercise were analysed by fast Fourier transform. We classified frequency bands according to previous studies (Kvernmo et al. 1999, Kvandal et al. 2006) into phase I (0.005-0.0095 and 0.0095-0.02 Hz), phase II (0.02-0.06 Hz: phase II) and phase III (0.06-0.16 Hz). The first peak of power spectra density (PSD) in SBF appeared at 0.0078 Hz in phase I. The second peak of PSD in SBF appeared at 0.035 Hz. The third peak of PSD in SBF appeared at 0.078 Hz. The first peak of PSD in DHb/Mb appeared at 0.0039 Hz, which was out of phase I. The second peak of PSD in DHb/Mb appeared at 0.016 Hz. The third peak of PSD in DHb/Mb appeared at 0.035 Hz. The coefficient of cross correlation was very low. Cross power spectra density showed peaks of 0.0039, 0.016 and 0.035 Hz. It is concluded that a peak of 0.016 Hz in oscillations of DHb/Mb observed in muscle during exercise is associated with endothelium-dependent vasodilation (phase I) and that a peak of 0.035 Hz in DHb/Mb is associated with sympathetic nerve activity (phase II). It is also confirmed that each peak of SBF oscillations is observed in each phase.

KEY WORDS: fast Fourier transform, cross power spectra density, endothelium-dependent vasodilation

INTRODUCTION

We found in a previous study that oxygenation of haemoglobin/myoglobin determined by using a near-infrared spectroscopy (NIRS) system oscillated in inactive skeletal muscle at rest as well as during exercise [22], and we decided to determine the origin of the oscillation. The amplitude of oscillation in deoxygenation in a resting state is enhanced by light exercise [23]. This suggests that energy metabolism increased by exercise affects the amplitude of oscillation of deoxygenation. The oscillation remains constant during light prolonged exercise [21]. This suggests that homeostasis of the body provides a constant environment in the muscle and consequently maintains oscillation of deoxygenation at a constant level. It is also known that oxygenation in skeletal muscle [23] and in the brain [4] oscillates in the intrinsic spot. Thus, although it is likely that local energy metabolism increased by exercise produces an oscillation of deoxygenation [5,20], the oscillation cannot occur within one cell, because NIRS should measure more signals than in one cell. Therefore, a physiological muscle system involving the oscillation should be supposed.

It is also known that there are oscillations in cutaneous blood perfusion in humans. Wavelet transform has been used for analysis

of signals of blood flow. It has been reported that the signal in the frequency interval from 0.0095 to 1.6 Hz consists of oscillations with five different characteristic frequencies. It has been shown that these oscillations may represent the influence of heart beat (1.6-0.6 Hz), respiration (0.4-0.16 Hz), intrinsic myogenic activity of vascular smooth muscle (0.16-0.06 Hz), neurogenic activity on the vessel wall (0.06-0.02 Hz) and endothelium-mediated vasodilation (0.02-0.0095 and 0.0095-0.005 Hz) [6,7]. Thus, direct physiological factors of oscillations in cutaneous blood perfusion have been studied in detail, though it is not clear why endothelium-mediated vasodilation has a rhythm.

Therefore, comparison of oscillations of skin blood flow and deoxygenation in skeletal muscle would make it possible to clarify the direct physiological factors of deoxygenation in skeletal muscle. However, an interrelationship between the original signal from energy metabolism [5,9,13,20] and the physiological factors that are determined from the comparison of skin blood flow and deoxygenation must be taken into consideration since measured deoxygenation is not obtained in a single muscle fibre but in a large portion of muscle (see Discus-

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sion). Thus, although it is necessary to discuss the interaction in a complex muscle system, we should first find a direct factor operating the oscillation of deoxygenation.

The purpose of the present study was therefore to compare the oscillation of skin blood flow with that of deoxygenation in muscle during light exercise in order to determine the physiological significance of oscillations in deoxygenation. We chose a light prolonged exercise in the supine position. The reason is as follows: Oscillation of deoxygenation in the muscle is enhanced immediately after the start of light exercise [20, 21], but it takes a long time for skin blood flow to attain a steady state level in light exercise [3,8]. If the increasing phase in skin blood flow is included, frequency analysis would be affected by the increasing phase. Furthermore, in the upright position, blood is pooled in the legs due to gravity. In order to reduce the effect of haemoglobin by the pooled blood and to enhance the effect of myoglobin on deoxygenation, we used the supine position.

MATERIALS AND METHODS

Eight healthy male volunteers participated in the present study. Their age, height and body weight were 20 ± 1.2 yrs, 173 ± 5.8 cm and 65 ± 8.1 kg, respectively. Consent for participation in the study was obtained from all subjects after informing them of the purpose of the experiment, the procedure, and possible risks. The study was approved by the local ethics committee.

Experimental protocol and determination of work rate

The experiment was carried out in the room temperature of 21°C. An electrically braked cycle ergometer (Combi 232C, Japan) controlled by a computer was used in the experiment. The subjects performed ramp exercise in a supine position to determine peak oxygen uptake (peak $\dot{V}O_2$). The power output was set at 20 watts for 1 min and was increased by 20 watts per minute until the subject was unable to maintain a revolution rate of 60 rpm.

From the linear relationship between oxygen uptake and work rate obtained in the ramp test, work rate in constant exercise was determined. This work rate corresponded to 50% peak oxygen uptake. After resting for 5 min in the supine position, constant exercise was performed in the supine position for 60 min with a revolution rate of 60 rpm.

Measurements

Skin blood flow (SBF) was measured using a laser blood flow meter on the right vastus lateralis muscle (Omega Flow FLO-N1, Omega Wave). A probe was attached to the right vastus lateralis muscle. Changes in SBF were determined during rest and exercise. Obtained analogue signals were recorded by a data recorder, with analogue-to-digital conversion at a sampling frequency of 1 Hz using MacLab/8s (AD Instruments, Castle Hill, Australia) for input into a personal computer.

Oxygenated haemoglobin/myoglobin (Hb/MbO₂) concentrations in the left vastus lateralis were measured using an NIRS system

(HEO200N, Omron, Japan). 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. Dual-wavelength light (760 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 depth of penetration of the radiation is about 1.5 cm [11]. The sampling frequency of Hb/MbO₂ was 1 Hz. NIRS is absorbed by haemoglobin and myoglobin. From changes in optimal densities (ΔOD), Hb/MbO₂, deoxygenated haemoglobin/myoglobin (DHb/Mb), and total haemoglobin/myoglobin (THb/Mb) were calculated by the following equations.

$$\Delta Hb/MbO_2 = \Delta OD(840 \text{ nm}) - 0.66 \cdot \Delta OD(760 \text{ nm}) \quad \text{----- (1)}$$

$$\Delta DHb/Mb = -0.59 \cdot \Delta OD(840 \text{ nm}) + 0.80 \cdot \Delta OD(760 \text{ nm}) \quad \text{----- (2)}$$

$$\Delta THb/Mb = 0.41 \cdot \Delta OD(840 \text{ nm}) + 0.14 \cdot \Delta OD(760 \text{ nm}) \quad \text{----- (3)}$$

Since DHb/Mb is a relative value, it cannot be used for comparison of DHb/Mb levels between different persons or between different regions of the body. However, data can be used for elucidating the time trend.

Ventilation and gas exchange responses were measured by an on-line computerized breath-by-breath method (AE-280S, Minato Medical Science, Japan). A 2-litre syringe was used to calibrate the system, which was linear throughout a range of 0-600 l·min⁻¹ of ventilation. Fractions of O₂ and CO₂ were analysed using a zirconium solid electrolyte oxygen analyser and an infrared carbon dioxide analyser, respectively. The gas analysers were calibrated by known standard gases (O₂: 15.0%, CO₂: 5.0%). Then oxygen uptake ($\dot{V}O_2$) was outputted for 15-s intervals.

Fast Fourier transform and statistics

Power spectra density (PSD) for deoxygenation was obtained by fast Fourier transform (FFT) with 3 windows and 50% overlap. Cross correlation (CR) between SBF and DHb/Mb in the muscle during exercise was obtained. Cross power spectra density (CPSD) was obtained between SBF and DHb/Mb in the muscle during exercise. PSD, CR and CPSD were obtained during constant exercise from 40 min to 50 min. Individual values of PSD and CPSD were normalized by individual highest value. The normalized values were averaged at the same frequency.

Figure 5 shows mean values and standard deviations.

Definitions

We divided the frequency band into three periods according to the reports by Kvernmo et al. [7] and Kvandal et al. [6]: Endothelium-mediated vasodilation (0.005-0.0095 and 0.0095-0.02 Hz) was termed phase Ia and phase Ib, neurogenic activity on the vessel wall (0.02-0.06 Hz) was termed phase II, and intrinsic myogenic activity of vascular smooth muscle (0.06-0.16 Hz) was termed phase III. A frequency band below phase III was not detected due to low frequency of sampling. Therefore, we did not use data above phase III.

RESULTS

Figure 1 shows an example of SBF (upper panel) and Hb/MbO₂, DHb/Mb and THb/Mb (lower panel). It seemed that SBF increased until 30-40 min and then showed a steady state in all subjects. There were some unexpected changes from the general trend in some subjects, but the SBF level seemed to show stability from 40 to 50 min. DHb/Mb abruptly increased and then showed a steady state or a slight decrease. On the other hand, Hb/MbO₂ and THb/Mb showed similar kinetics. After abrupt increases, Hb/MbO₂ and THb/Mb decreased and then showed a steady state. Thus, it seemed that the increasing phase after a sudden decrease in THb/Mb had no effect on DHb/Mb.

Figures 2 and 3 show individual data of SBF and DHb/Mb from 40 min to 50 min during exercise. There were oscillations in SBF. The amplitudes differed in subjects. There were also oscillations in DHb/Mb. There were differences in the amplitude of oscillation in DHb/Mb. However, DHb/Mb is not an absolute value but a unit of optical density. Therefore, we cannot compare these values among subjects. Oscillation of DHb/Mb was slower than that of SBF.

Figure 4 shows the coefficient in CR between SBF and DHb/Mb during exercise from 40 to 50 min. CR expresses the time trend of parameters. The coefficients were all very low, though there was a time shift for DHb/Mb. The results clearly showed that there was no time trend between SBF and DHb/Mb.

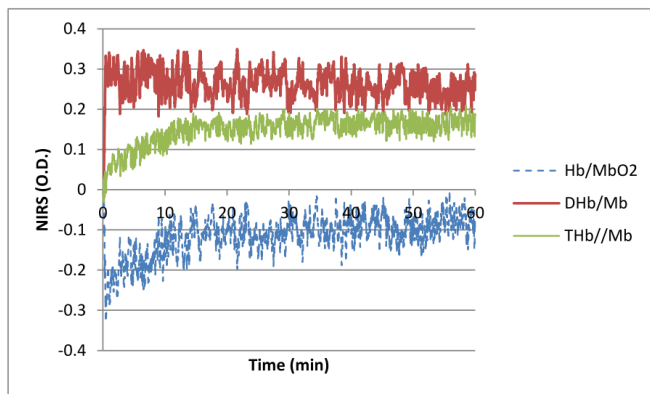
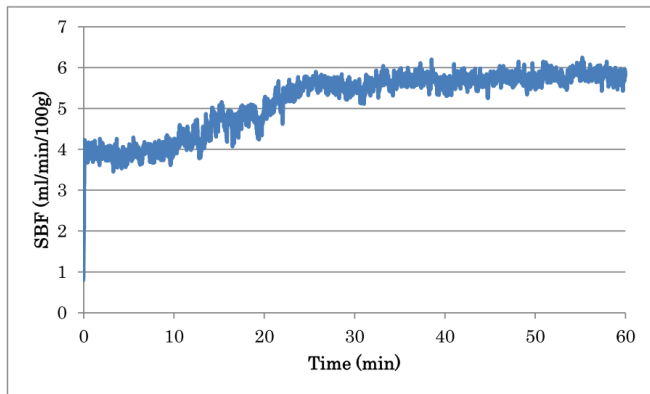


FIG. 1. THE UPPER PANEL SHOWS THE KINETICS OF SKIN BLOOD FLOW (SBF) DURING EXERCISE AND THE LOWER PANEL SHOWS OXYGENATION, DEOXYGENATION AND TOTAL HAEMOGLOBIN/MYOGLOBIN (Hb/MbO₂, DHb/Mb, and THb/Mb).

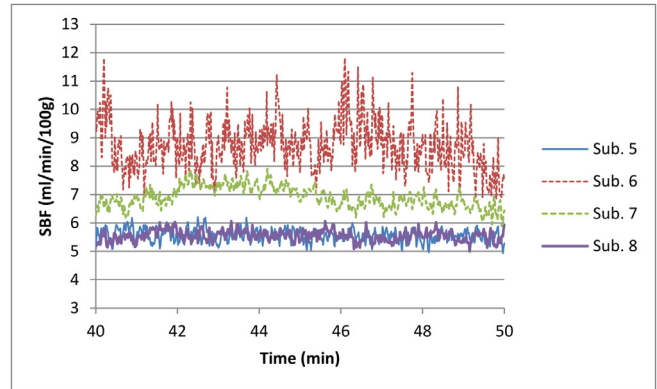
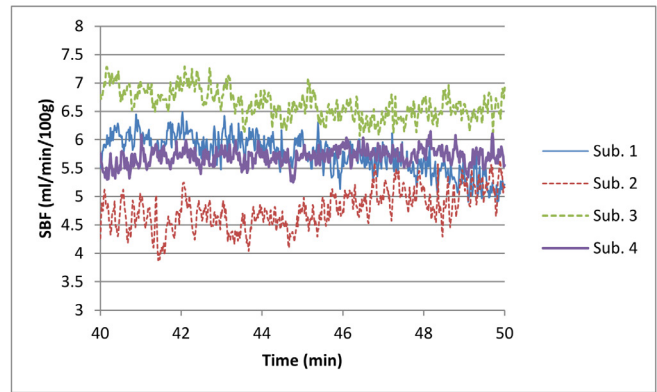


FIG. 2. SKIN BLOOD FLOW (SBF) IN ALL SUBJECTS FROM 40 MIN TO 50 MIN DURING EXERCISE. A COLOURED LINE SHOWS A CHANGE IN AN INDIVIDUAL VALUE.

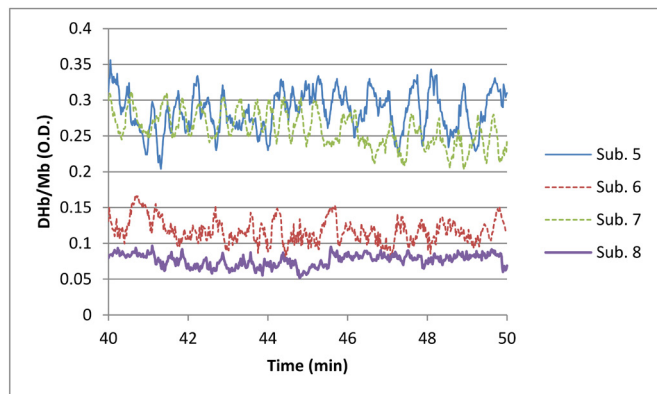
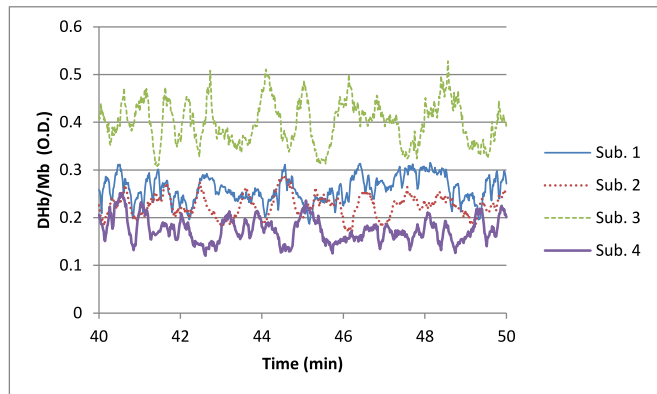


FIG. 3. DEOXYGENATION HAEMOGLOBIN/MYOGLOBIN (DHb/Mb) IN ALL SUBJECTS FROM 40 MIN TO 50 MIN DURING EXERCISE. A COLOURED LINE SHOWS A CHANGE IN AN INDIVIDUAL VALUE.

Figure 5 shows PSD in DHb/Mb and SBF obtained from 40 min to 50 min during exercise. In SBF, the first peak of PSD appeared at 0.0078 Hz in phase Ia. There was no peak in SBF in phase Ib. The second peak in SBF appeared at 0.035 Hz in phase II. The third peak in SBF appeared at 0.078 Hz in phase III.

The first peak of PSD in DHb/Mb appeared at 0.0039 Hz. This peak was slightly out of phase Ia. The second peak in DHb/Mb appeared at 0.016 Hz in phase Ib. The third peak in DHb/Mb appeared at 0.035 Hz in phase II. There was no increase in PSD in DHb/Mb in phase III.

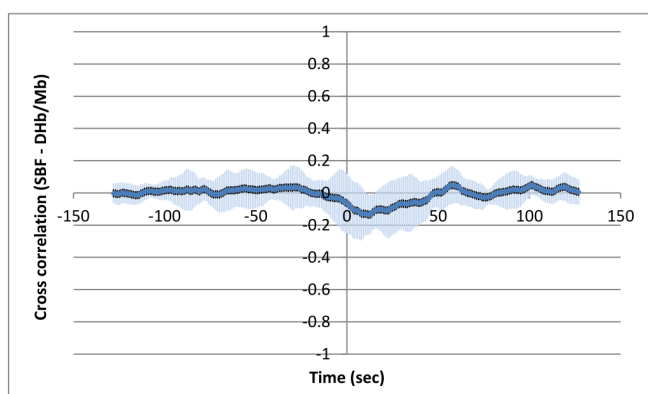


FIG. 4. CROSS CORRELATION BETWEEN SKIN BLOOD FLOW (SBF) AND DEOXYGENATION HAEMOGLOBIN/MYOGLOBIN (DHb/Mb).

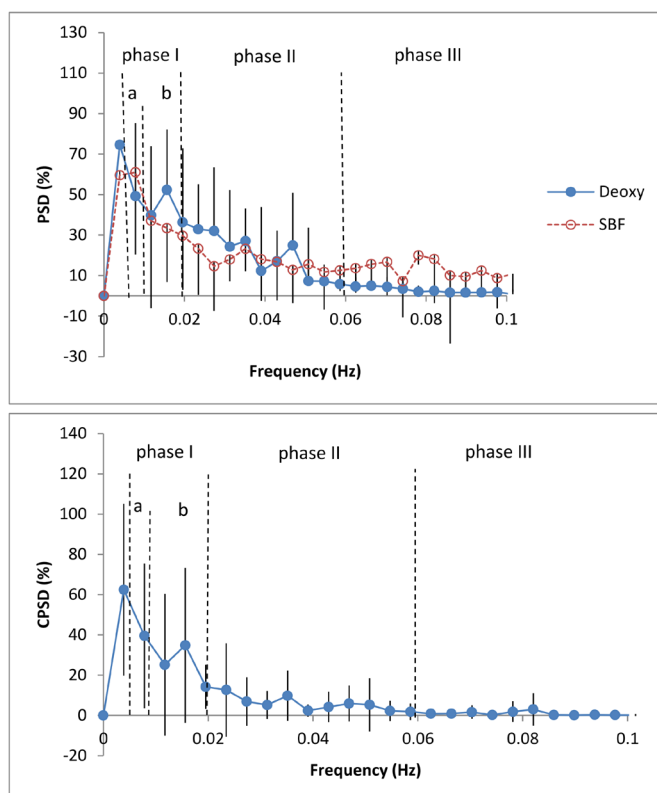


FIG. 5. AVERAGE POWER SPECTRA DENSITY (PSD) IN DEOXYGENATION HAEMOGLOBIN/MYOGLOBIN (DHb/Mb) AND SKIN BLOOD FLOW (SBF) FROM 40 MIN TO 50 MIN DURING EXERCISE. INDIVIDUAL VALUES WERE NORMALIZED BY AMPLITUDE OF THE HIGHEST VALUE IN PSD (UPPER PANEL).

Note: The normalized values were averaged in each frequency. Individual cross power spectra density (CPSD) between SBF and DHb/Mb in muscle from 40 min to 50 min during exercise was obtained. The obtained values were normalized by the highest value. The normalized values were averaged. Dotted lines show the division of phases.

CPSD, which expresses the frequency trend of parameters, also showed the first peak at 0.0039 Hz. This peak was slightly out of phase Ia. The second peak at 0.016 Hz was within phase Ib. There was a third peak at 0.035 Hz in phase II. There was no peak in phase III. The results showed that there was a frequency trend between SBF and DHb/Mb.

DISCUSSION

By moving from the sitting position to the supine position, blood can shift from the legs to the central circulation. It is therefore thought that the blood pooling in the legs is at a minimum before starting exercise, but due to an increase in muscle temperature caused by exercise, compliance of peripheral capacity vessels [8] is increased and consequently blood may be distributed to the legs [20]. An increase in THb/Mb due to venous blood pooling affected Hb/MbO₂. Therefore, the difference between Hb/MbO₂ and THb/Mb was determined in order to diminish the effect of blood pooling. The difference also becomes minus DHb/Mb (-Eq. (2)), which can be inferred from equations in the method (-Eq. (2) = Eq. (1) - Eq. (3)). For this reason, we used DHb/Mb instead of Hb/MbO₂.

Attention must be given to the effect of SBF on DHb/Mb and Hb/MbO₂ determined by NIRS. Hb/MbO₂ can be affected by the change in HbO₂ in skin blood. Results of previous studies have been contradictory, showing that Hb/MbO₂ determined by NIRS is not affected or is affected by SBF [1, 17, 18]. However, comparative analysis of ¹H-NMR and NIRS measurements showed that DHb/Mb obtained by NIRS expresses Hb/MbO₂ signals in skeletal muscle [18]. Furthermore, the present study clearly showed that the coefficient of CR between SBF and DHb/Mb was very low, suggesting that the time trend between SBF and DHb/Mb would be independent of each other.

There was a frequency trend between SBF and DHb/Mb. Two peaks of 0.016 Hz and 0.035 Hz in CPSD were observed in phase I and phase II, respectively. This shows that the physiological factor is the same in SBF and DHb/Mb.

A band of 0.035 Hz was obtained in CPSD in the present study. A study on cutaneous blood perfusion suggested that this band is derived from sympathetic nerve activity [16]. During exercise, SBF is increased due to vasodilation of arteries, probably by inhibition of sympathetic nerve activity. In handgrip exercise, it has been suggested that the level of sympathetic nerve activity to the skin is controlled by central command, while the level of sympathetic nerve activity to the muscle is affected by muscle afferent reflex [19]. However, in dynamic exercise, the level of muscle sympathetic activity that originates in active muscle is increased to more than 60% peak $\dot{V}O_2$ [14]. Thus, oscillations in SBF and Hb/MbO₂ in muscle may be induced by central command.

Acetylcholine (ACh) induces endothelium-independent vasodilation and sodium nitroprusside (SNP) induces endothelium-dependent vasodilation [6,7]. ACh and SNP increased the mean amplitude of the total spectrum. The only significant difference between the effects

of Ach and SNP was observed in the amplitude of oscillation with frequency of around 0.0095-0.02 Hz. Thus, the band of 0.0095-0.02 Hz in cutaneous blood perfusion is regarded as oscillation induced by endothelium-dependent vasodilation [7]. Recently, a band of 0.005-0.0095 Hz has also been reported to be related to endothelium-dependent vasodilation [6]. In the present study, there was a second peak in CPSD between SBF and DHb/Mb in phase Ib (0.016 Hz). This suggests that the frequency of DHb/Mb is derived from endothelium-dependent vasodilation.

Although it is well known that there is oscillation in SBF, it is still not clear why oscillation occurs. TCA and glycolysis are known to have oscillations [2,9,13]. Iotti et al. [5] reported that phosphocreatine (CrP) re-synthesis during recovery from light exercise oscillates at low frequency (0.002-0.025 Hz). Since CrP can recover with oxygen consumption, this frequency range means oscillation of oxygen consumption. Metabolic substances could transport upstream signals through endothelial cells and could induce remote vasodilation [12,15]. In this case, the oscillation due to a certain substance or some substances in the muscle could produce an oscillation of small arterial vessels. A small arterial vessel expands capillaries to some muscle fibres so that the oscillation of oxygen supply spreads to some activated muscle fibres. Eventually, the oscillations could affect the oscillation in muscle fibres. This means that oscillations in muscle fibres are synchronized. Thus, oxygen supply and oxygen consumption could interact with each other. Such a system is a closed muscle oxygen system.

The first peak in CPSD and PSD of DHb/Mb was found to be slightly out of phase Ia. It is known that an oscillation in CrP re-synthesis can be shifted by a change in cytosolic pH [5] and that the first peak of oscillation of DHb/Mb can be shifted by light exercise [20]. The frequency of an oscillation in CrP re-synthesis ranges from 0.002 to 0.025 Hz [5]. It may be possible that the range of Ia is expanded when oscillation occurs in the muscle oxygen system during exercise.

There was no peak of SBF in phase Ib in the present study. We used the division in band frequencies based on previous studies. Wavelet transform was used in those studies to analyse data of cutaneous blood perfusion, while we used FFT. Sampling frequency was very fast in the previous studies. This difference in the results between the previous studies and the present study might be due to the differences in the method used for analysis and in the sampling frequency.

CONCLUSIONS

A peak of 0.016 Hz in oscillations of DHb/Mb observed in muscle during exercise is associated with endothelium-dependent vasodilation. The oscillation could be a fundamental rhythm in a closed muscle oxygen system. A peak of 0.035 Hz in DHb/Mb is associated with sympathetic nerve activity. This stimulation could be derived from outside of a closed muscle oxygen system. It may be possible that phase Ia expands so as to include a peak of 0.0039 Hz in DHb/Mb during exercise. Iotti et al. [5] reported very slow oscillation ranging from 0.002 Hz to 0.025 Hz. It is also confirmed that each peak of SBF oscillations is observed in each phase.

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