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**COHERENCE BETWEEN OSCILLATIONS IN THE CARDIORESPIRATORY
SYSTEM AND IN TISSUE OXYGEN INDEX IN MUSCLE RECOVERING
FROM INTENSIVE EXERCISE IN HUMANS.**

Authors

Noboru Usuda¹ Kazuki Shirakawa ¹ Kei Hatano ¹ Masaki O. Abe² Takahiro Yunoki²
Tokuo Yano²

Affiliations

¹ Graduate school of Education, Hokkaido University, Kita-ku Sapporo, Japan

² Department of Human Developmental Sciences, Faculty of Education, Hokkaido
University, Sapporo, Japan

Short running title

Coherence between cardiorespiratory system and TOI

Abstract

It has been shown that the tissue oxygen index (TOI) measured by near-infrared spectroscopy oscillates at very low frequencies during recovery after exercise and that this oscillation is derived from interactions among biochemical substances involved in oxidative metabolism in skeletal muscle. As a further step, we examined whether TOI in muscle interacts through oscillation with factors related to oxygen in the cardiorespiratory system. For this examination, coherence and phase difference between the TOI in the vastus lateralis and heart rate (HR) and between TOI and arterial oxygen saturation (SpO₂) were sequentially determined during recovery (2-60 minutes) after severe cycle exercise with a workload of 7.5% of body weight for 20 seconds. Significant coherence between TOI and HR was obtained in the very low frequency band (approximate range: 0.002 Hz to 0.03 Hz) and in the low frequency band (approximate range: 0.06 Hz to 0.12 Hz). The phase difference was negative in the low frequency band and positive in the very low frequency band. The coherence between TOI and SpO₂ was significant in the very low frequency band. The phase difference was negative. There were no sequential changes in these coherences and phase differences. The results suggest that TOI in skeletal muscle interrelates with factors related to the heart and lungs. (211 words)

Key words

coherence, Cardiorespiratory system, Tissue oxygen index, oscillation, recovery from exercise

Introduction

Near-infrared spectroscopy (NIRS) is a method for noninvasively measuring tissue oxygen index (TOI). It has been reported that intramuscular TOI measured by this method oscillates during recovery from exercise (21). In that study, the power spectral density (PSD) of intramuscular TOI after exercise was calculated using fast Fourier transformation. The maximum PSD of TOI appeared in the very low frequency band (0.0039 - 0.0061 Hz) (21). Moreover, from evidence (7, 8) indicating that the process of re-synthesis of creatine phosphate accompanying oxidative phosphorylation oscillated in the very low frequency band (0.002 - 0.025 Hz) after exercise, it was suggested that TOI oscillates due to oxidative metabolism (21). Furthermore, it was pointed out by referring to Richards' oscillation model (15) that the oscillation of TOI was caused by feedback of ATP to oxidative phosphorylation (21). Recently, it has been reported that TOI oscillates in the very low frequency band (0.0039 - 0.0048 Hz) during exercise (20). In that report, in addition to the feedback process of ATP, it was pointed out that the interactions among biochemical substances of the TCA cycle cause oscillation of NADH and that TOI oscillates since NADH is involved in oxidative phosphorylation in the electron transport system (20). In fact, it has been observed that NADH oscillated when the TCA cycle was activated (1), though that observation was in an animal experiment. Thus, it has been shown that oscillation of TOI is derived mainly from oscillation related to oxygen metabolism in muscle fiber in the very low frequency band.

Since oscillation has the property of entrainment, if there is oscillation in each organ, it is possible for the oscillation to be propagated by entrainment. For example, the entrainment between muscle contraction rhythm and respiration rhythm has been studied for a long time (2, 14, 16). In addition, it is known that muscle pump action accompanying

muscle contraction during posture control affects circulation function (17). In other words, there seems to be an entrainment between muscle contraction and cardiorespiratory function. However, as far as we know, entrainment between the oscillation of oxidative metabolism in muscle and the oscillation of cardiorespiratory function has not been studied.

Meanwhile, intramuscular TOI reflects the balance between oxygen supply and consumption (3, 9). Therefore, there is also the possibility that the oscillation of oxygen supply affects the oscillation of TOI in muscle. Oxygen supply to active muscle depends on the product of cardiac output and arterial oxygen content (i. e., heart rate, stroke volume, arterial oxygen saturation, and arterial oxygen partial pressure). Therefore, these indicators are factors of the rhythm phenomenon of TOI in muscle. In addition to this, carbon dioxide (CO_2) produced by oxidative metabolism has the property of vasodilatation (12). Therefore, it can be deduced that end-tidal carbon dioxide partial pressure (PETCO_2) can cause TOI oscillation (22).

Thus, the oscillation of TOI is not a simple phenomenon derived from interactions among biochemical substances related to oxidative metabolism in skeletal muscle. This oscillation may possibly have a function of correlating with each organ of the respiratory and circulatory systems outside skeletal muscle due to entrainment. Therefore, in this study, we examined the interaction between the oscillation of TOI and the oscillation of factors related to oxygen in the heart and lungs.

Materials and Methods

Subjects. The subjects were 8 healthy men (age: 23 ± 2.4 years, height: 170.7 ± 8.1 cm, weight: 64.5 ± 10.3 kg). Each subject signed a statement of informed consent

following a full explanation regarding the nature of the experiment.

Study procedures. In this study, each subject performed an intensive exercise for 20 seconds on a cycle ergometer (PowermaxVII, COMBI, Tokyo, Japan) with a workload of 7.5% of body weight. Before the exercise, each subject rested on the cycle ergometer for 10 minutes. After the exercise, each subject got off the cycle ergometer and moved to a chair in order to decrease body motion and recovered for 60 minutes.

Measurement. Blood samples (each 100 μ l) were collected from fingers using a capillary tube. Each subject's hand was pre-warmed in 40 - 45 °C water while sitting on the ergometer in order to arterialized capillary blood (25). After this warming, the subject's hand was warmed by a heating glove at rest, during exercise and during recovery. It has been shown that such blood samples might not accurately reflect arterial O₂ pressure but can closely reflect arterial pH. Samples were analyzed using a blood gas analyzer (i-STAT, I-Stat, Abbon Point of Care Inc. IL. USA) to measure pH and lactate.

TOI was measured in the vastus lateralis by NIRS (NIRO200x, Hamamatsu Photonics, K.K. Hamamatsu, Japan) with a sampling rate of 1 Hz. The NIRS system consisted of irradiation and detecting devices, and the distance between the devices was 40 mm. Three different near-infrared rays (735, 810, 850 nm) from the irradiation devices penetrated into muscle, where they were scattered or absorbed in tissues and transmitted to the detecting device. In this study, we used spatially resolved spectroscopy (SRS). TOI was calculated by the ratio of oxy-hemoglobin (O₂Hb) and total hemoglobin (Hb) using the following formula:

$$TOI = \frac{O_2Hb}{Total\ Hb} \times 100 (\% TOI)$$

We measured arterial oxygen saturation (SpO₂) from the tip of a finger of the left hand using an oxygen saturation degree monitoring apparatus (PULSOX-300i, KONICA MINOLTA, Tokyo, Japan). The rates of absorption of red-color rays and infrared rays are different, and the oxygen saturation degree monitoring apparatus used the differences to estimate SpO₂.

PETCO₂ was measured by a respiratory gas analyzer (AEROMONITOR AE-310S, Minato Medical Science CO. LTD., Osaka, Japan) with the breath-by-breath mode. Expiration and inspiration flow rates were measured by a heat wire flowmeter. A 2.0 l syringe was used to calibrate the flowmeter. The CO₂ fraction was measured by an infrared absorption analyzer, and the O₂ fraction was measured by a magnetic dumbbell analyzer. To calibrate these sensors, we used standard density gas (O₂: 15.38%, CO₂: 5.132%). Heart rate (HR) was measured by a heart rate monitor connected to the respiratory gas analyzer.

Data analysis. HR and PETCO₂ breath-by-breath data were converted to 1 second data by the interpolation method.

Data obtained from 2 to 60 minutes during recovery were used for analysis. Data for the first 2 mins after exercise were not used for analysis because subjects used that time for getting off the cycle ergometer.

All signals were filtered using a band pass (0.001 Hz - 0.2 Hz) to focus on the low frequency oscillation which is shown to relate with oxygen metabolism in preceding studies (7, 8, 21). Data for analysis consisted of oscillation around the axis and level of

the axis of oscillation. Trend of the level of axis was removed using the detrend function in Matlab software.

Coherence analysis and phase analysis. Coherence analysis was carried out to determine the common frequency characteristics between two signals over time between 2 minutes and 60 minutes during the recovery phase. In this study, the coherence between TOI and other items (HR, SpO2, PETCO2) was calculated. Data of 1,500 points was taken as the basis of the discrete Fourier transform (DFT). We split this DFT into 3 parts with 512 samples of a Blackman window. In addition, while maintaining the 1,500 points, by sequentially moving 100 DFT points along the recovery time axis, time series DFT (R_{xy}) during recovery was obtained.

$$|R_{xy}(\lambda)|^2 = \frac{|f_{xy}(\lambda)|^2}{f_{xx}(\lambda) f_{yy}(\lambda)},$$

where λ is frequency, $f_{xx}(\lambda)$ and $f_{yy}(\lambda)$ represent each signal, and f_{xy} is a cross spectrum.

The phase difference was calculated by the cross spectrum.

Statistics Results are presented as means $\pm$ standard deviations. Significant levels of blood pH and blood lactate from resting values were tested by Dunnett's method. The significant level was set at $p < 0.05$. The following numerical expression was used to determine the significant level ($p < 0.05$) of coherence and phase difference:

$$|R_{xy}(\lambda)|^2_{1-\alpha} = 1 - \alpha^{1/(Ne-1)}$$

where Ne represents the degrees of equivalent freedom, and α represents the statistically

significant level.

Ethics

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.

Results

The maximum power with the cycle ergometer was 671.9 ± 11.6 watts. Table 1 shows blood pH and lactate at rest and during recovery. Blood lactate significantly increased after exercise and then decreased toward the resting level. Blood pH was also significantly decreased after exercise. The blood pH recovered to the resting value.

Figure 1 shows typical data for TOI, HR, SpO₂ and PETCO₂ during recovery from exercise. TOI rapidly decreased approximately 10 seconds after the start of exercise and increased rapidly immediately after stopping exercise. Then TOI recovered to the resting value and oscillated. HR increased after the start of exercise and decreased rapidly during recovery. During the recovery phase, HR oscillated. SpO₂ decreased slightly in the exercise phase. In the recovery phase, SpO₂ recovered to the resting value and then oscillated at that time. PETCO₂ was maintained at the resting value in half of the subjects and decreased rapidly after the start of exercise in half of the subjects. Then, after rapidly increasing, PETCO₂ decreased to the resting value and then oscillated at that time.

The following results were obtained by analysis of data obtained between 2 and 60 minutes during the recovery period.

Figure 2 shows the results for TOI-HR coherence. TOI-HR coherence was statistically significant between the lower limit of about 0.06 Hz and the upper limit of about 0.12 Hz (low frequency band), and the phase difference of the band was negative. TOI-HR coherence was also statistically significant between the lower limit of about 0.002 Hz and the upper limit of about 0.03 Hz (very low frequency band). The phase difference of the band was positive. Figure 3 shows the results for TOI-SpO₂ coherence. There was significant coherence between the lower limit of about 0.002 Hz and the upper limit of about 0.03 Hz. The phase difference of the band was negative. Figure 4 shows the results for TOI-PETCO₂ coherence. There was significant coherence at various frequencies, but it was scattered. The phase difference of the band was not clear due to the scattered results.

Discussion

TOI oscillation affected by Dual factors. NIRS is a noninvasive way to measure muscle blood flow and oxygenation (4). It has been shown that muscle oxygenation measured by NIRS using the Beer-Lambert method (BLM) oscillates (24). However, oxygenation measured by the BLM is affected by skin blood flow (11). Skin blood flow has very low frequency components (10). Therefore, oscillation of TOI determined by spatial resolution spectroscopy (SRS), which is not affected by skin blood flow rate, has been confirmed in the very low frequency band (20, 21).

TOI in skeletal muscle reflects the balance between oxygen supply and oxygen consumption (3, 9). For example, a decrease in the level of TOI (decline in the axis of oscillation) is observed after the start of exercise. This is because oxygen consumption

exceeds oxygen supply. On the other hand, immediately after the exercise, it is possible to observe an overshoot of the axial level of TOI in skeletal muscle (elevation of the axis of oscillation) because oxygen supply exceeds oxygen consumption. However, these explanations are not applicable to the rising and falling phases observed by the oscillation of TOI. This can be understood by the following simple model (23). First, TOI is expressed by the difference between oxygen supply and oxygen consumption. Next, the oscillation of oxygen supply is represented by a sine curve. Similarly, the oscillation of oxygen consumption is represented by a sine curve having a phase difference from the oxygen supply. Then the oscillation of TOI, which is the difference between them, becomes a sine curve. When the level of each axis of oscillations of oxygen consumption and oxygen supply is changed, the difference between the two axis levels appears as a change in the axis level of TOI. Since the level of the axis of TOI decreases during exercise, it can be deduced that the change in the level of oxygen consumption is greater than the level of oxygen supply. Even in this case, there is oscillation of TOI, although it is difficult to observe in a short exercise such as that in present study. From this point of view, the overall image of the oscillation of TOI should be reviewed from the relationship between oxygen supply and consumption.

Influence of cardiorespiratory system on TOI (from HR, SpO₂ and PETCO₂).

Oxygen supply to active muscles is determined by cardiac output and arterial oxygen content. Cardiac output is the product of stroke volume and heart rate, and arterial oxygen content is the sum of hemoglobin-bound oxygen and amount of oxygen dissolved in the blood. Arterial oxygen content reflects gas exchange in the lungs.

The power spectral density of human heart rate fluctuation has been found to

have components in the low frequency band (less than 0.12 Hz) and in the high frequency band (0.224 to 0.28 Hz) (23). The high frequency band is mediated by the parasympathetic nervous system. However, this frequency band was not examined in this study, and we therefore cannot discuss it. Also, low-frequency components have not been observed for oxidative metabolism related to the recovery phase of TOI or creatine phosphate. Therefore, it is likely that the coherence in the low frequency band found in this study indicates the existence of propagation from the heart to TOI of skeletal muscle through oscillation of cardiac output

There was significant coherence between TOI and SpO₂ in the very low frequency band. Although SpO₂ is a value related to arterial oxygen content and is a value determined in the lung, owing to a value at the fingertip, there is a time lag from oxygen saturation in the lung. Therefore, the time difference between SpO₂ determined at the fingertip and TOI determined in the lateral vastus muscle corresponds to the delay time. However, since SpO₂ reflects the value of arterial blood, this item should be involved in oxygen supply. Therefore, it is thought that propagation of SpO₂ oscillation from the lungs to skeletal muscle exists in the very low frequency band.

The effect of CO₂ accumulation by artificially manipulating respiratory resistance on oxygenation of the brain and active muscles has been investigated (12). As a result, it has been observed that oxygenation rose at both sites as PETCO₂ increased. It was also suggested that these increases in oxygenation were derived from an increase in blood flow. However, this effect is derived from the level of CO₂ and not from the CO₂ oscillation. Therefore, we cannot directly link this result with the results of this study

Yano et al. (22) proposed from evidence of cross correlations of pulmonary ventilation, heart rate and PETCO₂ that the dynamic homeostasis of PETCO₂ is

maintained as follows: 1) there is a feedback signal via the carotid artery of PETCO₂, 2) CO₂ is excreted by ventilation, and 3) CO₂ is transported to the lung due to cardiac output. Also, the result of these interactions seems to induce PETCO₂ oscillation. Furthermore, it was inferred that oscillation of PETCO₂ produced oscillation of oxygenation. However, the results of this study suggest that the oscillation of PETCO₂ has little effect on the oscillation of intramuscular TOI.

Influence of TOI on heart. We stated in the introduction that 1) there was the possibility that TOI oscillated in relation to oxidative phosphorylation of the electron transfer system, that 2) the recovery process of creatine phosphate after exercise oscillated and its recovery was due to oxidative phosphorylation, and that 3) the oscillation of TOI after exercise was related to oxygen consumption. Moreover, it was an important point that the oscillations described in 2) and 3) occurred in very low frequency bands. This suggests that TOI of the very low frequency band is related to oxygen consumption in skeletal muscle fibers (20, 21). Therefore, oxygen consumption probably oscillates within skeletal muscle fibers. As a further step in this study, it was shown that the TOI oscillation is propagated to the heart.

In physiology, we are interested in the mechanism by which the relationship between TOI and HR is established. In this study, it is physiologically significant that TOI oscillation propagated to HR oscillation. Since we examined results obtained after exercise, the signal output from muscle mechanical receptors (19) or central command (5) would have had little effect on HR. The muscle metaboreflex mainly affects HR (See references (6, 13, 18, 22) for muscle metaboreflex, though oscillations of metabolites are not described in those reports.). In this study, there are some points to note about

metaboreflex. The oscillation was distinguished from the axial level of oscillation and the influence of this axial level was removed in this study. As a result, in the recovery period after the exercise, the TOI oscillation had coherence with the HR oscillation despite a change in the level of the muscle metabolism. Therefore, this coherence is basically an entrainment between them. In addition, the relationship between muscle metaboreflex and HR level does not seem to be completely clear unless consideration is given to changes in the level of TOI. However, the entrainment at a very low frequency found in this study is a new finding that will be valuable for examining the mechanism of muscle metaboreflex.

Interaction in the respiratory circulatory systems (centering on TOI). It is possible to reexamine the relationship between each of the physiological items described above from the phase difference. However, it should be noted that the phase difference does not always represent a causal relationship. For example, when the phase difference is 90 degrees, there may be a phase difference of -270 degrees. Although there are such restrictions, it is one of the useful tools for examining the causal relationship.

In the very low frequency band, the phase difference between TOI and HR was negative. This means that TOI precedes HR. Therefore, it can be interpreted from the phase difference that HR influences TOI in the low frequency band. Furthermore, since the phase difference of SpO₂ was negative, it can be concluded that SpO₂ affects TOI. These relationships are consistent with what has been discussed above.

As mentioned above, TOI influenced HR. At the same time, the oxygen supply system (HR and SpO₂) affected TOI. HR is a function of the heart pumping and SpO₂ is an element determined in the lungs. Also, it is thought that the oscillation of TOI itself is

caused by the interactions among biochemical substances of the oxidative metabolism in muscles and by the oscillation of oxygen supply. The oscillation of this TOI seemed to have interactions with the oscillation of elements of the respiratory circulation system. These interactions may involve order formation in the cardiorespiratory system, but further examination will be necessary to elucidate this point.

Conclusion. The coherence of TOI and HR and that of TOI and SpO₂ did not change with time regardless of the metabolic change after exercise. The oscillation of each item should be distinguished from the magnitude of axial oscillation. An influence of oscillation related to oxygen supply on TOI oscillation was found in the low frequency band (HR - TOI) and the very low frequency band (TOI - SpO₂). Oscillation of TOI related to oxygen consumption in skeletal muscle affected HR in the very low frequency band. These results suggest that TOI in skeletal muscle is interrelated with factors related to the heart and lungs.

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Table

Table 1. Mean Value and standard deviation (SD) of arterial blood pH and blood lactate at pre-exercise and post exercise.

	pre	post-5min	post-15min	post-30min	post-60min
pH	7.39±0.02	7.21±0.03 [†]	7.26±0.03 [†]	7.32±0.03 [†]	7.37±0.02
Lac ⁻ (mmol/L)	1.09±0.24	12.77±1.45 [†]	11.63±1.42 [†]	7.62±1.24 [†]	3.01±0.38 [†]

[†]: significant differences from pre-exercise.

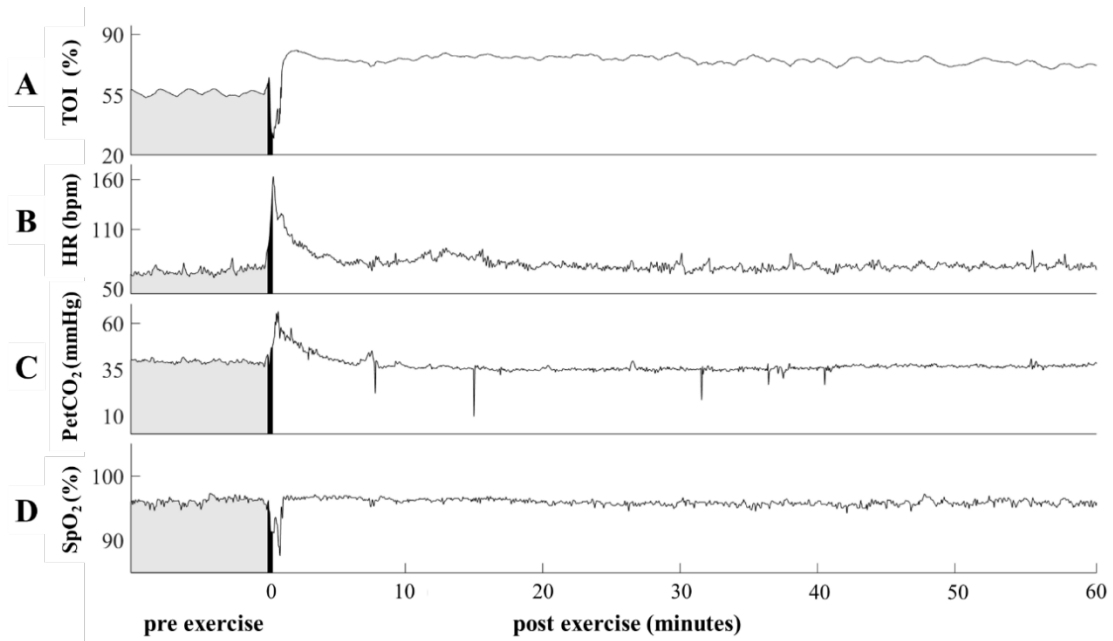


Figure 1. Data for one subject. Raw data of A) TOI in the vastus lateralis, B) HR, C) SpO₂, and D) PETCO₂ for entire time (rest: 10 minutes, exercise: 20 seconds, recovery: 60 minutes). The gray area represents rest, the black area represents exercise, and the white area represents recovery.

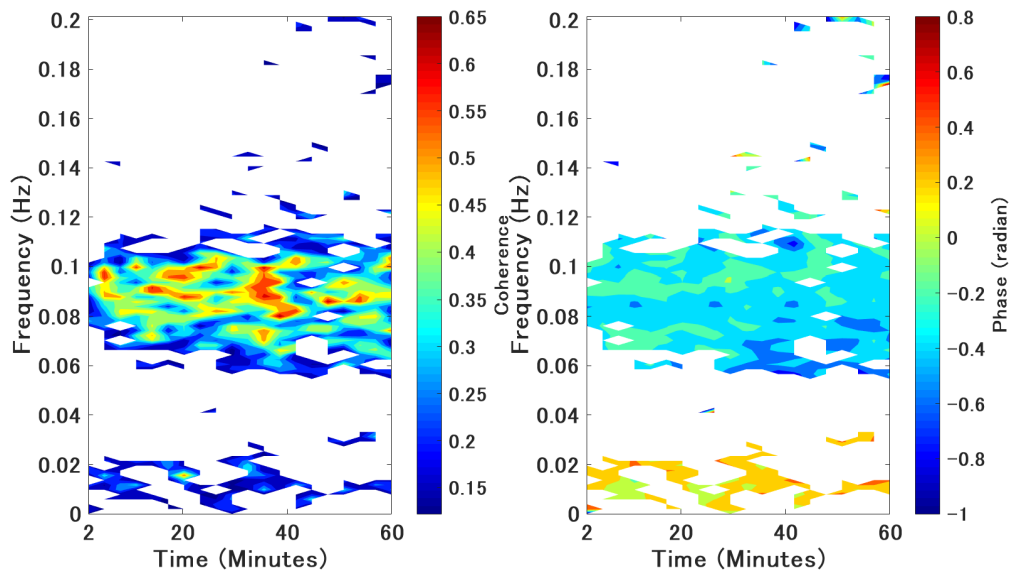


Figure 2. Pooled data from eight subjects during recovery (2-60 minutes). A) Pooled TOI-HR coherence and B) pooled TOI-HR phase difference are shown. The coherence scale is represented in a color scale (the highest is red, the lowest is blue and no significant coherence is white). The phase scale is represented in a color scale (no phase difference is zero, the positive highest phase difference is red, and the negative highest difference is blue).

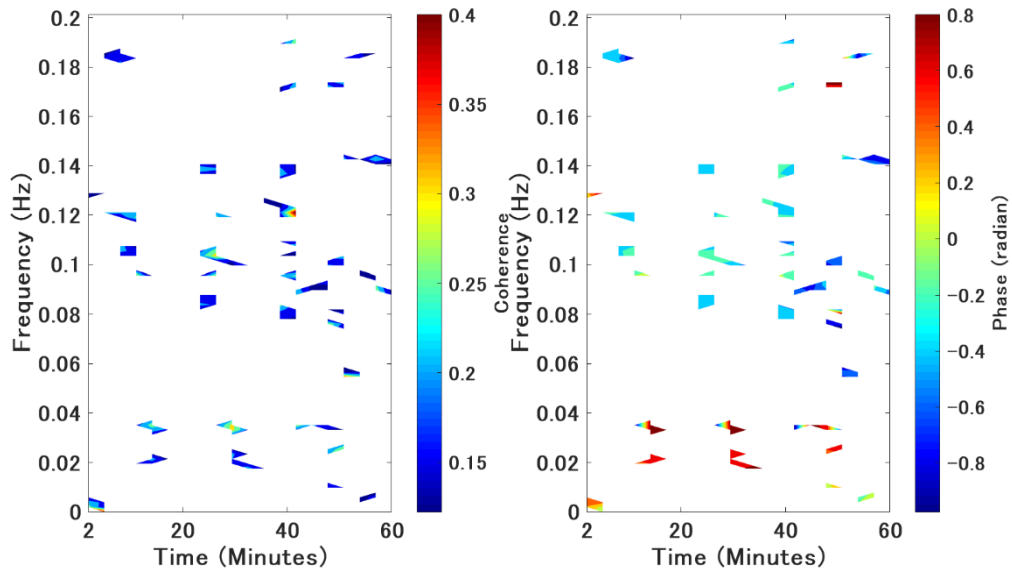


Figure 3. Pooled data from eight subjects during recovery (2-60 minutes). Pooled TOI-PETCO₂ coherence and pooled TOI-PETCO₂ phase difference are shown. The coherence scale is represented in a color scale (the highest is red, the lowest is blue and no significant coherence is white). The phase scale is represented in a color scale (no phase difference is zero, the positive highest phase difference is red, and the negative highest difference is blue).

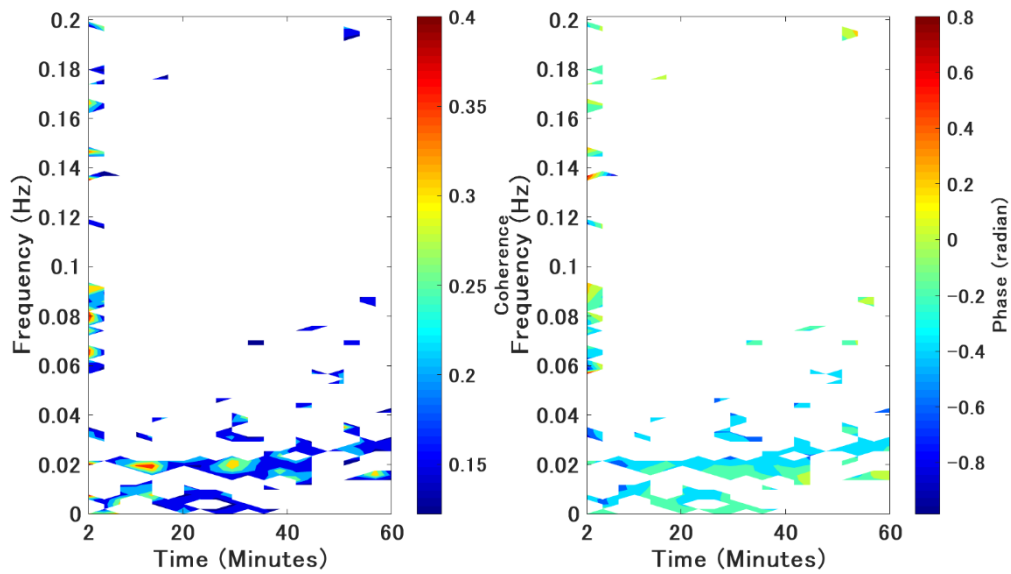


Figure 4. Pooled data from eight subjects during recovery (2-60 minutes). Pooled TOI-SpO₂ coherence and pooled TOI-SpO₂ phase difference are shown. The coherence scale is represented in a color scale (the highest is red, the lowest is blue and no significant coherence is white). The phase scale is represented in a color scale (no phase difference is zero, the positive highest phase difference is red, and the negative highest difference is blue).