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Evaluation of Muscle Oxygenation during Exercise by NIRS in Normal Subjects—Significance of the NIRS Threshold—

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Abstract: The purposes of the study were to indentify values for TOI threshold and deoxy-hemoglobin threshold as well as TOI slope using a new NIRS measurement in the vastus lateralis muscle during exercise, and to evaluate the clinical significances in the NIRS variables. To check the reproducibility of NIRS parameters, ten healthy males participated in preliminary two tests with a separate 1-week interval. Another fifteen healthy males participated in the study. Mean values of TOI slope and wattage at TOI threshold did not differ significantly in two tests. The mean values of TOI slope was –0.115 ± 0.07%/watt, and the mean wattage of HHb threshold, TOI threshold, and THI peak were 194 ± 40 watt, 188 ± 36 watt, and 135 ± 35 watt, respectively. Post-hoc testing showed that the workloads at the NIRS threshold were significantly greater than at VT, LT, and THI peak. The workload at TOI threshold was significantly correlated with those at VT, peak THI, and peak watt, but not with those at BMI and LT. These results suggest that NIRS threshold reflects the capacity for oxygen exchange in muscles, thus affecting exercise performance in healthy subjects. (J Jpn Coll Angiol, 2007, **47**: 21–27)

Key words: near-infrared spectroscopy, exercise performance, tissue oxygenation index (TOI) slope, NIRS threshold

Introduction

Oxygen is transported to peripheral muscles by the cardiocirculatory system, and is changed into energy, which is used in exercise. The volume of oxygen uptake ($\dot{V}O_2$) is used to evaluate exercise capability. In addition, anaerobic threshold (AT) and lactate threshold (LT) are generally available for exercise prescription as indices of exercise load as well as heart rate. Although the capacity for aerobic endurance activity is usually assessed by maximal O_2 uptake ($\dot{V}O_2$max), ventilatory threshold (VT),[1] or by LT,[2] these parameters are all systemic metabolic variables.

The lower limb muscles of a person exercising every day have adapted to exercise, and exhibit minimal fatigue during exercise. However many sedentary individuals stop exercising due to muscle fatigue and discomfort, before the limit of cardio-pulmonary function. It is thus necessary to devise an index for exercising muscles with which exercise abilities in sedentary, elderly, and physically normal subjects can be evaluated. Although lactic acid production from exercising muscles is used as an index for anaerobic metabolism, drawing blood to measure is invasive and this cannot be routinely performed.

It has been reported that near-infrared (NIR) spectroscopy can be used as a non-invasive method of evaluating the state of oxygenation in skeletal muscle.[3] Differences in absorption characteristics of oxy-hemoglobin (O_2Hb) and deoxy-

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Table 1 Summary of subjects

	Study 1	Study 2
Case no	10 (M)	15 (M)
Age (y.o)	25.0 ± 4.7	25.3 ± 6.9
Height (cm)	172.9 ± 2.7	170.7 ± 4.1
Body mass (kg)	69.3 ± 5.1	63.5 ± 4.8
Body mass index ($kg \cdot m^{-2}$)	23.1 ± 1.8	21.4 ± 1.4
Watt at VT (watt)	94.7 ± 22.5	95.4 ± 22.3
Watt at LT (watt)	NT	97 ± 24
Maximal watt (watt)	248.2 ± 30.9	249 ± 37
Maximal $\dot{V}O_2$ (ml/kg/min)	35.4 ± 4.3	35.5 ± 4.3

hemoglobin (HHb) in the near-infrared spectrum (NIRS) have been found useful to estimate relative changes in local muscle oxygenation, blood volume, the dynamics of local muscle O_2 supply, and muscle O_2 consumption in real time during exercise.[4–9]

Muscle O_2Hb level measured by NIRS gradually decreased while workload in normal subjects increased.[10] Especially, the slope of tissue oxygenation index (TOI) was steeper in chronic obstructive pulmonary disease (COPD) patients than in age-matched normal subjects, and the TOI-slope in COPD patients was related to body mass index (BMI).[11] Furthermore, we have reported that the slope of muscle oxygenation reached nadir, and plateaued despite an increase in workload.[12]

Approaches to measurement with NIRS have progressed, and have shifted from time spectrum method to spatially spectrum methods. In particular, a newly developed form of NIR spectroscopy called spatially resolved (SR) spectroscopy has enabled non-invasive and continuous measurement of muscle oxygen saturation during exercise.[13] The accuracy of SR spectroscopy has been proven by measuring tissue phantoms and has been validated in measuring human skeletal muscle by comparison with data obtained with time resolved spectroscopy.

The purposes of this study were to measure TOI threshold and deoxy-hemoglobin threshold as well as TOI slope using this new NIRS method in the vastus lateralis muscle during exercise, and to evaluate the clinical significances of these NIRS variables.

Materials and Methods

Subject 1

Ten healthy males {age: 25.0 ± 4.7 yr, height: 172.9 ± 2.7 cm, BMI: 23.1 ± 1.8 $kg \cdot m^{-2}$} participate in the test for reproducibility of NIRS parameters during incremental exercise.

Subject 2

Fifteen active healthy males {age: 25.3 ± 6.9 yr (19–43 yr), height: 170.7 ± 4.1 cm, BMI: 21.4 ± 1.4 $kg \cdot m^{-2}$} without competitive cycling experience participated in this study (**Table 1**).

They were required to avoid high-intensity exercise and training for 3 days prior to the test. In addition, they were required to have a light meal at least 1 hour before the test. The subjects were informed of the potential risks involved before signing a written voluntary consent form in accordance with the policy statement of the American College of Sports Medicine. Since the voluntary consent for blood sampling could not be obtained from the 6 subjects, blood sampling was conducted on the 9 subjects who had signed the form. Institutional approval of the study was obtained from the Graduate School of Medicine, Osaka City University.

Exercise protocol

Study 1

Each subject performed two incremental exercise tests at a one-week interval. The incremental exercise was conducted until exhaustion using the same protocol and the ramp meth-

ods.

Study 2

Each subject performed an incremental exercise to reach exhaustion on a cycling ergometer (Corival, Lode, B.V. Medical Technology Groningen, The Netherlands).

Their respiratory gas exchange and NIRS data were recorded at rest and during exercise. The exercise protocol consisted of 2 min resting, 3 min warm-up exercise (0 W, 60 rpm), and incremental cycling exercise. Maximal cycling exercise was started from 0 W at 60 rpm and gradually increased at a rate of 15 W/min until exhaustion, which was defined as failing to maintain a pedal rate of 60 rpm.

$\dot{V}O_2$ and CO_2 production ($\dot{V}CO_2$) were measured by the breath-by-breath method using a gas analyzer (AE-280, Minato Medical Science, Osaka), and recorded in a computer with a sampling rate of every 2 seconds. Maximal oxygen consumption ($\dot{V}O_2max$) was confirmed by meeting the 2 of the following 3 criteria,[14]: 1) leveling off or increase of less than 150 ml in $\dot{V}O_2$ despite increase in a work rate; 2) respiratory exchange ratio greater than 1.10; and 3) attainment of heart rate higher than 95% of the age-related maximum (220 – age). VT was determined by the conventional v-slope method, and the respiratory compensation segments of the exercise test were excluded from this analysis.[1]

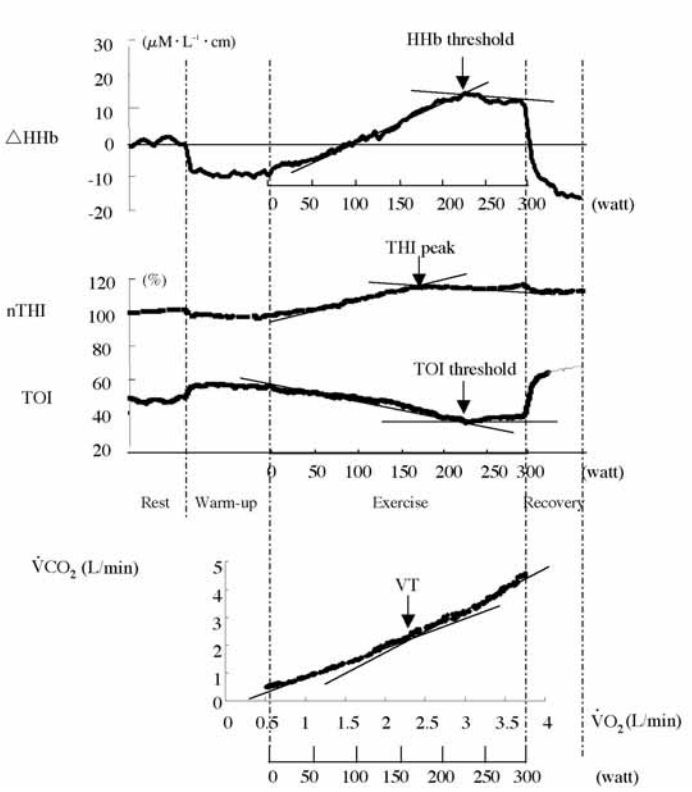

Figure 1 Trends in three NIRS variables (deoxy-haemoglobin: ΔHHb, tissue oxygenation index: TOI, and normalized tissue hemoglobin index: nTHI) measured from the right vastus lateralis muscle at rest, warm-up, and during and after incremental cycling exercise in a representative subject by comparison with ventilatory threshold (VT).

Two milliliter of brachial venous blood was obtained from 9 subjects at rest and every 2 min during incremental cycling exercise. [La-] was determined by an enzymatic method (ESAT 6661 Lactate, Eppendorf, Germany).

NIR spectroscopy

The NIRS apparatus (NIRO-200: Hamamatsu Photonics K. K., Hamamatsu) utilized in this study employs a 3-wavelength continuous wave system, combining the modified Beer-Lambert and SR spectroscopy methods. Changes in O_2Hb and HHb were measured by focusing on the differences in absorption of light at 775, 810, and 850 nm. The slope of the NIR light attenuation versus distance relation was measured at a point distant from the light input, from which the absolute ratio of O_2Hb to total Hb content, making it possible to calculate semi-quantitatively both average TOI and total Hb content (THI), using photon diffusion theory.[15]

Changes in NIRS variables and VT in a representative case at rest and during exercise are shown in **Fig. 1**. With increase in workload after the first 2–3 min of incremental exercise, NIRS values began to exhibit consistent changes.

TOI began to decrease gradually with graded exercise. The decrease in TOI was linear fashion with respect to increase in work rate (TOI slope), and subsequently TOI reached nadir. The inflection point of TOI, indicated by arrows in **Fig. 1**, at which flattening of slope occurred, was determined by iteratively fitting different combinations of two regressions lines. This inflection point of TOI is defined as TOI threshold. HHb and THI gradually increased with graded

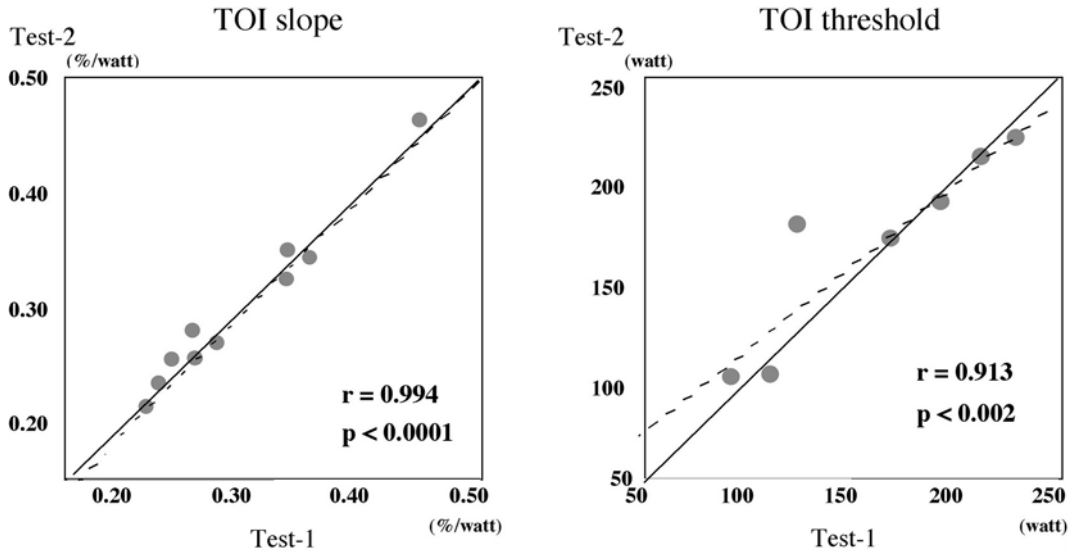

Figure 2 TOI slope and TOI threshold showed high correlations respectively between test-1 and test-2.

exercise, and finally reached plateau as well as TOI. The inflection point of HHb is defined as HHb threshold, at which flattened of HHb occurred. THI increased linearly until it peaked (THI peak), and then leveled off at the end of exercise. We focused on determining both TOI and HHb thresholds, at which these slope reached plateau (**Fig. 1**).

During measurement, changes in HHb was expressed in μmol・L^{-1}・cm with respect to an initial value (at steady-state at rest) arbitrarily set equal to zero, TOI was expressed in %, and THI was normalized to the initial value set equal to 1 (100%). The raw NIRS data were measured with a sampling frequency of 0.5 Hz, and were smoothed by the moving average of 5 data points.

Statistics

Values are presented as the mean ± S.D. Equality of variance assumptions were examined in advance by a Bartlett test for each variable. One-way factorial ANOVA was used to examine the significance of differences among variables for VT, LT, and NIRS, with Fisher's PLSD test performed for post-hoc analysis. Correlations between the variables at VT, LT, HHb threshold, TOI threshold, and THI peak were examined by identifying Pearson's correlation coefficients. Findings of $p < 0.05$ were considered significant. Analyses were performed using DA Stats Macintosh and Stat View Windows version 5.0.

Results

(1) Reproducibility of NIRS parameters

The TOI slope was evident in all 10 subjects, and the TOI threshold was observed in 7 of 10 subjects. Mean values of TOI slope and watt at TOI threshold in test-1 did not differ significantly from those in test-2, and both variables showed a significant correlation ($r = 0.99$, $p < 0.0001$, $r = 0.91$, $p < 0.002$) between test-1 and test-2, respectively. The regression lines obtained for correlations TOI slope and TOI threshold were $y = 0.02 + 1.03x$ and $y = 31.52 + 0.83x$, respectively. No significant differences in either intercept or coefficient between the regression line and identity line were found for either TOI slope or TOI threshold (**Fig. 2**).

(2) Physiological responses at VT and maximal exercise

The mean values of power output at VT and maximal watt and $\dot{V}O_2$/kg are shown in **Table 1**.

(3) NIRS parameters and comparison of exercise parameters

The mean values of TOI slope was –0.115 ± 0.07%/watt.

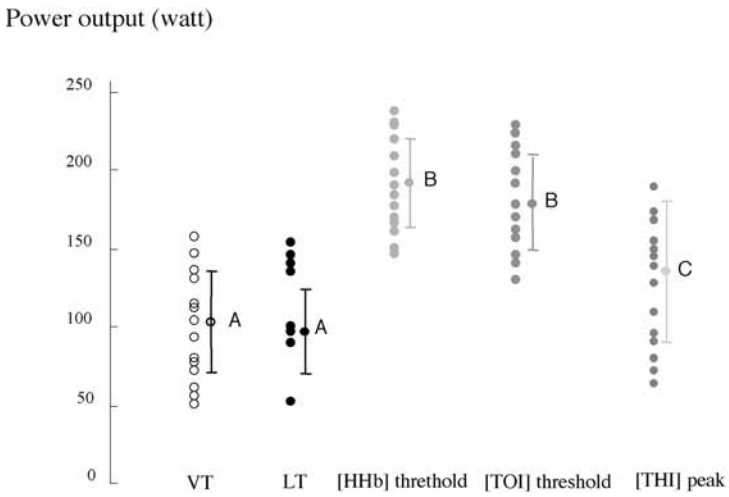

Figure 3 Comparison between power output at VT, LT, and various NIRS variables.
A vs B: $p < 0.0001$, A vs C: $p < 0.01$, B vs C: $p < 0.0001$

HHb threshold, TOI threshold, and THI peak were 194 ± 40 watt, 188 ± 36 watt, and 135 ± 35 watt, respectively. One-way factorial ANOVA revealed significant differences between workload ($p < 0.001$) at VT, LT, and at the 2 NIRS thresholds. Post-hoc testing showed that the workloads at the HHb threshold and TOI threshold were significantly greater ($p < 0.001$, all cases) than at VT, LT and THI peak (**Fig. 3**).

TOI slope was positively correlated with BMI, but not significantly correlated with VT, LT, THI peak, or peak watt. In contrast, the workload at TOI threshold was significantly correlated with those at VT, peak THI, and peak watt ($r = 0.73$, $p < 0.01$; $r = 0.72$, $p < 0.01$; $r = 0.79$, $p < 0.001$, respectively), but was not with those at BMI and LT (**Table 2, Fig. 4**).

Discussion

In the present study, TOI measured in healthy subjects gradually decreased with graded exercise, and the rate of decrease in TOI was significantly correlated with BMI. TOI flattened at 188 ± 36 watt regardless of increase in workload. This inflexion point of TOI (TOI threshold) was the same as the deoxy-Hb (HHb threshold). We defined these inflexion points as the NIRS threshold, which was related to ventilatory threshold, peak total hemoglobin volume in exercising muscles, and maximal watt. These findings show that NIRS index including TOI slope and NIRS threshold in exercising muscles affects exercise capacity as well as indices of cardio-pulmonary function.

In measuring oxygenation levels with NIR spectroscopy in the lower extremities, the scape between skin and a muscle affects measurement accuracy. We integrated NIRS with a central distance between the light source and photodiode of 4 cm. The influence of adipose tissue thickness (ATT) on NIRS propagation in leg muscles and on the sensitivity of the NIRS instrument have been investigated by an ultrasound examination, which demonstrated that NIR light penetrates shallow regions of muscle even when ATT is 1.5 cm.[16] In the present study, the average BMI was 21.4 ± 1.4 $kg \cdot m^{-2}$, marking below the normal value. The ATT of our subjects appeared sufficient in permitting NIRS penetration to measure O_2 dynamics (the change or balance between oxy-Hb and deoxy-Hb within relatively deep muscle). In addition, even though myoglobin (Mb) has the same absorption spectra as Hb, the ratio of [Hb] to [Mb] in human skeletal muscle is > 5, suggesting that NIRS signals originate mainly from Hb.[17]

The other problem with the NIRS method is the reproducibility of changes in TOI slope and TOI threshold. In degree of decrease in NIRS, there was no significant difference between 2 tests conducted at an interval of 1 week. In addi-

Table 2 Correlations of TOI slope and THI threshold with BMI, VT, LT, THI peak, and maximal work load

	BMI	VT	LT	[THI] peak	peak watt
TOI slope	$p < 0.05$	NS	NS	NS	NS
TOI threshold	NS	$p < 0.01$	NS	$p < 0.01$	$p < 0.001$

tion, flattening of TOI developed in 7 of 10 subjects in both tests, and the TOI thresholds in 2 tests indicated an insignificant difference. These findings demonstrate the reproducibility of TOI slope, the appearance of TOI threshold, and values of TOI threshold during incremental exercise. These NIRS parameters, thus, serve to evaluate exercising muscle.

In this study, the inflexion point of TOI, which was defined as TOI threshold, was the same as that of deoxy-Hb. Changes in TOI represent a dynamic balance between O_2 supply and O_2 consumption in the small vessels such as the capillaries, arteriolar, and venular beds.[9] It has been thought that the TOI threshold indicates the capacity for oxygen exchange from oxy-Hb to deoxy-Hb in muscle. Therefore, when oxygen exchange in hemoglobin reaches a limit during exercise, oxygen consumption in muscles cannot increase by oxygen exchange at a hemoglobin interval. However, many normal subjects were able to continue exercise above the TOI threshold. It appears that the increase in oxygen uptake in exercising muscle above the TOI threshold is maintained by increase in amount of blood flow to exercising muscles.

Peripheral circulation in the lower limb muscles strongly affected exercise ability. We reported that blood flow in the femoral artery measured by echography gradually increased during endurance exercise in normal subjects.[18] In the present study, blood flow in the calf was not measured, though increase in blood flow to exercising muscle is clearly important in maintaining exercise.

As another factor of blood supply to peripheral muscles, blood volume in exercising muscles also affects exercise ability. Blood volume is indicated by THI on NIRS measurement. THI gradually increased with increase in graded workload in this study. The increase in THI during exercise was thought to be the increase in blood volume in local muscles with activation of the cardio-circulatory system

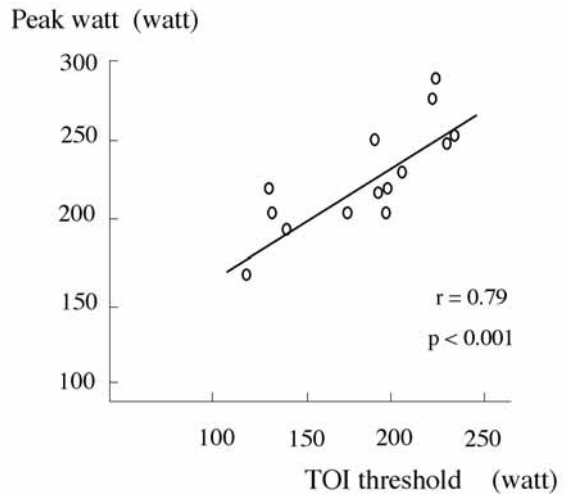

Figure 4 Correlation of peak watt and TOI threshold. Each symbol represents the value for one subject.

throughout the entire body and vasodilatation of active muscle. In this study, THI peaked at 134 ± 35 watt, and flattened afterwards. This peak THI was significantly related to NIRS threshold, and shows that the capacity for oxygen exchange of Hb in a muscle is related to the rate of increase in blood volume in peripheral muscle. This finding indicates that the NIRS threshold generally reflects the blood volume in very small arteries, capillaries, and very small veins in active muscle.

In this study, both NIRS threshold and VT were related to maximal exercise performance. In healthy subjects, the limit of oxygen exchange in hemoglobin in a muscle strongly affected exercise ability. Muscle's oxygen exchange ability correlates with VT and exercise ability: the higher the limit of muscle's oxygen exchange ability, the higher VT and exercise ability.

Limits of measurement with NIRS are that it is derived

from hemoglobin oxygenation level in a limited region of muscle, and NIRS is not influenced by amount of blood flow in exercising muscle. It will thus be important to examine peripheral muscle during exercise by the methods of NIRS measurement as well as index of amount of blood flow in the lower limbs.

Conclusion

We examined the changes in TOI and THI with the NIRS methods in the vastus lateralis muscle during exercise. Our results suggest that TOI slope functions as an index of capacity for oxygenation in exercising muscles, and that it affects the quantity of blood flow in lower limb muscles. In addition, NIRS threshold might reflect the capacity of oxygen exchange in muscles, and might affect the exercise performance in healthy subjects. Thus, these NIRS parameters provide information on exercising muscles, and will prove useful in the fields of training and rehabilitation.

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