Non-invasive quantitative assessment of oxidative metabolism in quadriceps muscles by near infrared spectroscopy

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Abstract

***Background*—Near infrared spectroscopy can be used in non-invasive monitoring of changes in skeletal muscle oxygenation in exercising subjects.**

***Objective*—To evaluate whether this method can be used to assess metabolic capacity of muscles. Two distinctive variables abstracted from a curve of changes in muscle oxygenation were assessed.**

***Methods*—Exercise on a cycle ergometer was performed by 18 elite male athletes and eight healthy young men. A measuring probe was placed on the skin of the quadriceps muscle to measure reflected light at two wavelengths (760 and 850 nm), so that the relative index of muscle oxygenation could be calculated. Exercise intensity was increased from 50 W in 50 W increments until the subject was exhausted. During exercise, changes in muscle oxygenation and blood lactate concentration were recorded. The following two variables for assessment of muscle oxygenation were then abstracted and analysed by plotting curves of changes in muscle oxygenation: the rate of recovery of muscle oxygen saturation (R_R) and the relative value of the effective decrease in muscle oxygenation (D_{eff}).**

***Results*—Data analysis showed a correlation between muscle oxygenation and blood lactate concentration at the various exercise intensities and verified the feasibility of the experiment. Data for the athletes were compared with those for the controls using the Aspin-Welch test of significance; t = 2.3 and 2.86 for R_R and D_{eff} respectively. There were significant differences (p = 0.05) between the athletes and the control group with respect to these two variables.**

***Conclusion*—R_R and D_{eff} may be distinctive variables that can be used to characterise muscle oxidative metabolism during human body movement.**

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Keywords: recovery; muscle; oxygen saturation; exercise; elite athletes

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Near infrared spectroscopy (NIRS) is widely used to monitor oxygen distribution in the intact brain and muscle tissue of humans and animals,[1–3] especially non-invasive monitoring of changes in human skeletal muscle oxygen in exercising subjects.[4 5] Our work is based on the findings of our predecessors. Primarily, the levels of muscle oxygen measured by NIRS are only understood as the result of the dynamic balance between muscle oxygen delivery and consumption. In the evaluation of muscle energy metabolism during exercise, other investigators have emphasised the variable half recovery time of muscle oxygen (T_R) after exercise. The use of T_R avoids difficulties of quantifying changes in oxyhaemoglobin (HbO_2) and provides a comparable variable for evaluating oxidative metabolism in muscles of different subjects.[6] Other investigators have also shown that, because of the significant correlation with regulatory metabolites of oxidative phosphorylation (ADP and phosphocreatine), the rate of decline in O_2 in ischaemia immediately after exercise determined by NIRS can be used to quantitatively evaluate localised muscle oxidative metabolism.[7] These studies were based on the results of simultaneous measurement, using NIRS and magnetic resonance spectroscopy, in finger flexor muscles during arterial occlusion immediately after exercise.[8] The results suggest that the rate of decrease in muscle oxygen may be a variable that could be used to evaluate oxidative metabolism in muscle.

The purpose of this study was to explore characteristic variables that could be used to assess oxidative metabolism in quadriceps muscle using NIRS in a subject performing incremental cycle ergometer exercise. Two groups of subjects were used: elite athletes and healthy volunteers. The experimental protocol and defining variables were carefully explained. On the basis of parametric statistics of the data obtained, two distinctive variables were selected that show significant differences in muscle metabolism.

Methods

SUBJECTS

Twenty six male subjects (aged 19–23, mean weight 67.3 kg) were recruited from among students of Beijing University of Physical Education, 18 of whom were elite athletes and the others healthy volunteers. Table 1 shows the events and personal bests of the elite athletes.

INSTRUMENT AND DETECTION

The non-invasive NIRS measurements were performed with a commercially available NIRS

Table 1 Events and personal bests of the elite athletes

n	*Event*	*Personal best*
4	100 m race	11 s
4	400 m race	50 s
5	800 m race	1 min 58 s
2	5000 m race	16 min
2	10 km walking race	44 min 15 s
1	50 km walking race	4 h

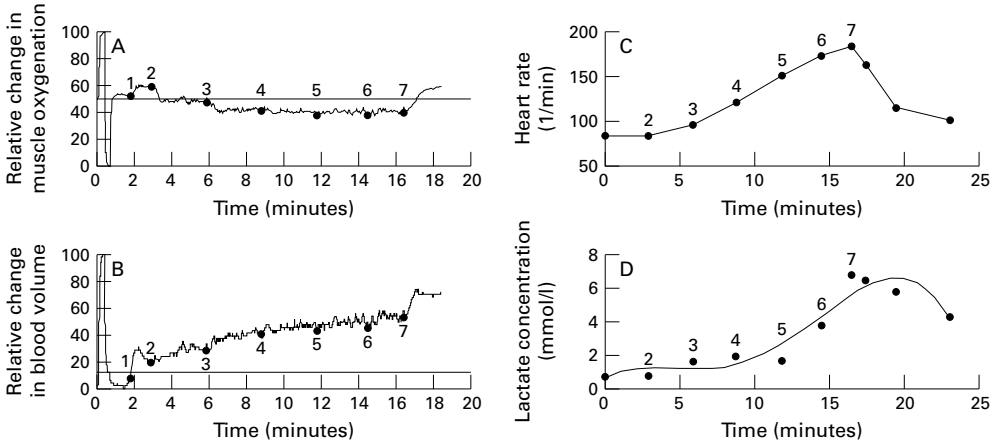

Figure 1 Comprehensive measurement of multiple variables during exercise on a cycle ergometer for an elite 800 m runner. (A) Muscle deoxygenation; (B) blood volume; (C) heart rate; (D) lactate concentration. Markers 1–7 indicate the 50 W incremental steps of exercise intensity.

unit (Runman; NIM Incorporation, Philadelphia, PA, USA; developed by B Chance and coworkers). The optical probe of the instrument was placed on the skin over the middle part of the vastus lateralis. A tungsten lamp embedded in the optical probe illuminated the tissue, and a pair of silicon diode detectors with optical filters for the different wavelengths (760 nm and 850 nm) were used to measure reflected light at these two wavelengths. The distance between the source and the detector was 4 cm. Light absorption of HbO_2 in the muscle tissue was higher than that of Hb at 850 nm and lower at 760 nm. The difference in reflected light intensity between 760 nm and 850 nm provided a measure of change in muscle oxygen saturation, and the sum of the two values provided a measure of change in blood volume. The output signal of the instrument was sampled by a personal computer through a custom developed A/D converter at a sampling rate of 3 Hz, and the relative values of oxygen saturation and blood volume were displayed on the screen in real time. Each subject was required to sit on the cycle ergometer and asked to warm up for one minute (from marker 1 to marker 2 in fig 1) and then begin the test. Warm up is defined as maintaining 50 W complete pedal turns per minute with a slack brake belt. An incremental series of loads in graded steps was tested for each subject. Exercise intensity started at 50 W, with 50 W step increments until the subject was exhausted. The duration of each step was three minutes so that most of the subjects could finish five steps. The last exercise step was stopped when the subject reached a state of exhaustion, regardless of whether or not this level of exercise was completed. Blood lactate concentrations and heart rates were also measured at the end of every step to produce a comprehensive analysis of oxidative metabolism. Arterialised capillary blood (15 µl) was taken from the earlobe of each subject when at rest and during the last 5–10 seconds of each workload. Lactate concentration was determined by an enzymatic method. Heart rates were monitored using a pulse watch made by Seiko.

DATA HANDLING AND STATISTICAL PROCEDURES

$\overline{X}_1$ and $\overline{X}_2$ are the means of the variables used to assess oxidative metabolic capacity of the athletes and controls respectively, and SD_1 and SD_2 the sample standard deviations. Because the population standard deviation of each variable for the athletes and the controls ($\sigma_1$and σ_2) was not known, the Aspin-Welch test was used to check if there was a significant difference between $\overline{X}_1$ and $\overline{X}_2$.[9] The t value of the Aspin-Welch test is given by:

$$t = (\overline{X}_1 - \overline{X}_2)/\sqrt{(SD_1/n_1) + (SD_2/n_2)} \quad (1)$$

where n_1 and n_2 are the sample sizes for the athletes and controls respectively. The critical value of t is written as $t_{df,p}$, where p is the significance level and df the degrees of freedom. From the Aspin-Welch test and Satterthwaite approximation,[10] we obtain:

$$df = (k_1 + k_2)^2/(k_1^2/(n_1-1) + k_2^2/(n_2-1)) \quad (2)$$

where $k_1 = SD_1/n_1$ and $k_2 = SD_2/n_2$. The value of $t_{df,p}$ can be obtained from the ordinary t table (two sided test). If the statistical result for a variable is $t > t_{df,p}$, it suggests that there is a significant difference between the athletes and controls in that variable.

Results

RESTING STATE HEART RATE

To confirm the difference in physique between the athletes and controls, resting heart rates

Table 2 Two sample, two sided Aspin-Welch test (p=0.05) comparing elite athletes with controls

Variable	*Athletes (n=18)*	*Controls (n=8)*	*t*	*df**	$t_{df,\,0.05}$**	*Conclusion*
Resting heart rate (H_R) (1/s)	63.3 (4.9)	73.6 (10.00	3.05	8.5	2.28	Significant difference
Half recovery time of muscle oxygen saturation (T_R), (s)	29.4 (7.15)	32.8 (8.0)	0.93	12.2	2.18	No significant difference
Half recovery increment of oxygenation saturation (h) (au)	6.5 (1.5)	5 (1.6)	2.24	12.5	2.17	Significant difference
Recovery rate of muscle oxygenation (R_R) (1/s)	0.225 (0.085)	0.165 (0.045)	2.3	24.3	2.06	Significant difference
Relative value of effective fall in muscle oxygen (D_{eff}) (au)	5.82 (4.26)	10.37 (3.05)	2.86	16.5	2.12	Significant difference

Values are mean (SD).
df, degrees of freedom for Aspin-Welch test; $t_{df,\,0.05}$, critical values of t (p=0.05) obtained from ordinary t table (two sided test); au, arbitrary units.

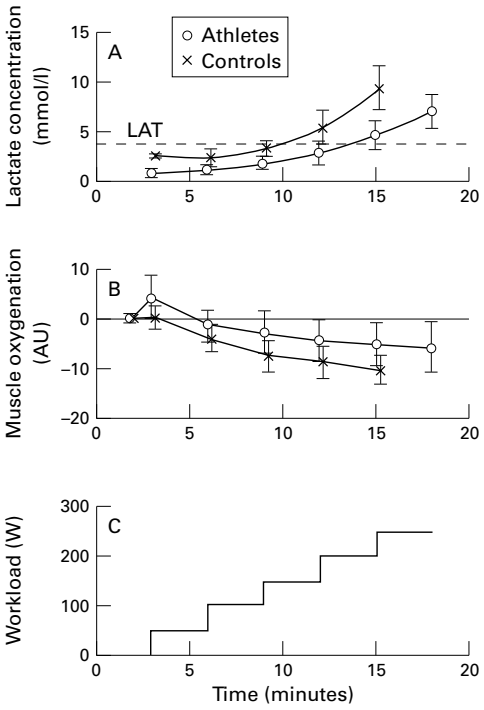

Figure 2 Correlation between blood lactate and muscle oxygenation at various exercise loads. Experimental results of exercise on a cycle ergometer for quadriceps muscle of elite male athletes (n = 18) and controls (n = 8). After a one minute warm up, exercise intensity was increased from 50 W in 50 W step increments until exhaustion. Changes in lactate concentration (A) and muscle deoxygenation (B) were recorded. The loads resulting in exhaustion for the elite athletes and controls were more than 250 W and 200 W respectively. (C) Incremental loads of cycle ergometer. LAT, Lactate threshold.

were measured (table 2). The athletes had significantly lower heart rates than the controls.

COMPREHENSIVE MEASUREMENT OF MULTIPLE VARIABLES

Muscle oxygenation, blood volume, blood lactate concentration, and heart rate were recorded during incremental level load exercise. The measurements provided multiple variables for analysing the course of oxidative metabolism. Typical responses of muscle deoxygenation, blood volume, blood lactate concentration, and heart rate are given in fig 1 for an elite 800 m runner. Muscle oxygenation decreased gradually with the increase in load (fig 1A), showing that the balance between muscle oxygen delivery and consumption changed with the exercise load. Blood volume (fig 1B) and heart rate (fig 1C) increased with increases in exercise load, showing that the blood circulated more rapidly and the ratio of blood entering the muscle to total blood increased.

Figure 2 shows the correlation between blood lactate concentration and muscle oxygenation at various exercise intensities. Some important phenomena can be observed. Firstly, the fall in muscle oxygen is less in athletes than in controls at all loads. Secondly, at the end of the warm up periods, there are upward overshoots of muscle oxygen, so that its mean in athletes (4.6 (4.3)) is larger than in controls (0.5 (2.2)). These two findings remain during recovery after exercise. Thirdly, below and above the blood lactate threshold (LAT; work intensity corresponding to 4 mmol/l), the mechanism of muscle energy metabolism during exercise can be divided into two modes: below the LAT, aerobic metabolism plays a dominant role in energy supply, and in this workload range muscle oxygenation falls rapidly and lactate concentration increases relatively smoothly; above the LAT, lactate concentration increases rapidly and muscle oxygen tends to be saturated, which shows that anaerobic metabolism is dominant.

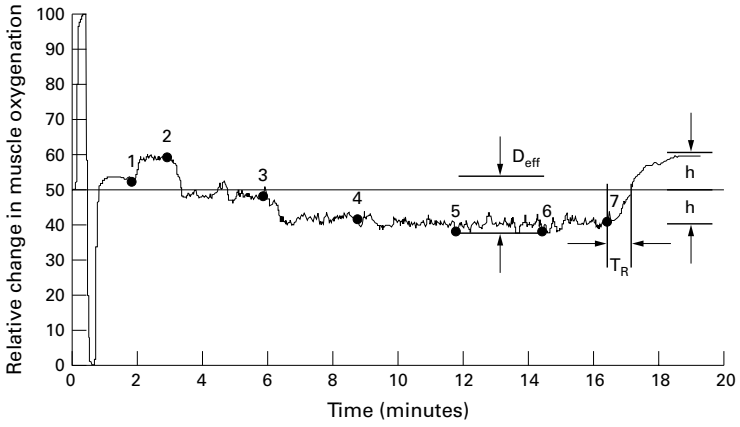

Figure 3 Definition of D_{eff} and T_R for assessment of metabolic capacity from the curve of changes in muscle deoxygenation.

HALF RECOVERY TIME OF OXYGEN SATURATION (T_R), HALF RECOVERY INCREMENT OF OXYGEN SATURATION (H), RECOVERY RATE (R_R), AND RELATIVE VALUE FOR THE EFFECTIVE DECREASE IN MUSCLE OXYGEN (D_{EFF})

To define these variables, a typical muscle deoxygenation curve (fig 1A) was amplified (fig 3). After the one minute warm up, tissue oxygenation gradually decreased with the exercise load, which was increased through 50, 100, 150, 200, and 250 W (markers 2–7). The subject was in a state of exhaustion at the end of the 250 W level, so the experiment was stopped at this point. After cessation of exercise, muscle oxygenation recovered and reached a higher level than in the resting state before exercise (the period before marker 1). This is called the overshoot recovery of muscle oxygenation. If h was half the increment of muscle oxygenation during the recovery period from the point of exhaustion, then T_R, which is the time taken to reach the level of h from the point of exhaustion, is defined as half recovery time. The recovery rate is defined as $R_R = h/T_R$, which describes the speed of recovery of muscle oxygenation after the cessation of exercise. D'_{eff} is defined as the decrease in muscle oxygen from the quiet state to the end of the 200 W load, which was close to saturation, as shown in fig 3. Considering the differences in individual body weight, we modified D'_{eff} as follows:

$$D_{eff} = D'_{eff} \times (\text{individual body weight/mean body weight of group}) \quad (3)$$

According to the above definitions, the data of h, T_R, R_R, and D_{eff} for each subject were obtained from the corresponding muscle deoxygenation curves; the mean values $\overline{X}$ and sample standard

deviation SD of h, T_R, R_R, and D_{eff} for the two groups were also calculated (table 2). Using equation (1), the value of *t* for h, T_R, R_R, and D_{eff} was obtained (table 2). The degrees of freedom and coefficient k were calculated using equation (2). The values in table 2 are the critical *t* values for p = 0.05. According to the Aspin-Welch test comparing *t* and $t_{df,0.05}$, we found that there was a significant difference for h, R_R, and D_{eff} between the elite athletes and controls. For T_R the mean value for athletes was lower than for the controls, but the difference was not significant. As R_R, which is equal to h/T_R, includes both h and T_R, we suggest that R_R and D_{eff} may be characteristic variables that can be used to assess muscle oxidative metabolism during human body movement.

Discussion

This study is based on the comprehensive measurement of multiple variables. The concept of LAT was used to examine what happens to muscle oxygenation below and above this value. The changing trends in muscle oxygenation and blood lactate concentration during incremental exercise loads agree with the theories of aerobic and anaerobic metabolism. Similar conclusions presented previously and their physiological significance can now be explained in detail. Previous workers[10 11] showed that measured muscle oxygen saturation does not represent changes in blood oxygenation in a single vessel but a weighted average of the saturation of arterial, capillary, and venous HbO_2 and intercellular oxymyoglobin. Arterial HbO_2 saturation does not normally change as work rate is increased. From the dynamics of the change in muscle oxygen saturation of the venous blood, it appears that the major desaturation, measured by NIRS, is due to oxygen loss from haemoglobin for work rates below the LAT and from myoglobin above the LAT.

In assessment of oxidative metabolism in muscles by NIRS, both R_R and D_{eff} should be taken into account. We suggest that R_R, which is equal to h/T_R, is the best variable to use to characterise muscle oxidative metabolism, because it is directly proportional to h and inversely proportional to T_R. Table 2 shows that the mean of h is larger and that of T_R smaller for the elite athletes than for the controls, so there are two factors that influence R_R in the same direction.

D_{eff} could be another candidate for assessing muscle metabolism. If two subjects with the same weight were asked to bear the same load in a cycle ergometer test, then the decreases in muscle oxygenation should be comparable. If the decrease in one was greater than in the other, in order to maintain the balance between oxygen consumption and delivery, muscle oxygenation would be maintained at a lower level in the former. To evaluate oxygenation in subjects of different weight, a modified factor must be considered. The *t* value of D'_{eff} was 2.86 if the modified factor was taken into account, which is more significant than the *t* value of 2.37 if the modification was not considered.

Adipose thickness of the subject is the main factor influencing the sensitivity and accuracy of the near infrared tissue oximeter. Because most of the subjects to be evaluated have the same build and adipose thickness (range measured by diagnostic ultrasound 5–8 mm), for an appropriate source-detector distance (4 cm in our work), the higher sensitivity and lower error would be used.[12] Another problem is that there is a difference between the sizes of the two groups; inclusion of more control data would be useful.

In summary, the results of this study validate a potential method for non-invasive quantitative evaluation of oxidative metabolism in muscles by near-infrared spectroscopy. They suggest that the recovery rate of muscle oxygenation, R_R, and the relative value for the effective decrease in muscle oxygen, D_{eff}, may be used as characteristic variables. This method should have applications in various research areas, including athlete training, rehabilitation, and sports medicine.

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Take home message

A non-invasive near infrared spectroscopy technique was used to assess the oxidative metabolic capacity of skeletal muscle. Rate of recovery of muscle oxygen saturation and the effective decrease in muscle oxygen at a given exercise load may be distinctive variables for characterising muscle oxidative metabolism during human movement.