Noninvasive determination of exercise-induced vasodilation during bicycle exercise using near infrared spectroscopy

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Summary

Background: The purpose of this study was to examine the changes in total hemoglobin (Δ[tHb]) response during bicycle exercise at various constant workloads using near infrared continuous wave spectroscopy (NIRcws) in humans. We hypothesized that the Δ[tHb] during exercise may progressively increase as a result of a dilation of the vascular bed and/or capillary recruitment at lower constant work rates.

Material/Methods: Seven healthy subjects performed bicycle exercise at 20, 40, 60, 80, and 100% of maximal work rates (Wmax) for 5 min. Muscle oxygenation change (Δ[Oxy]) and Δ[tHb] at the right vastus lateralis were monitored using a NIRcws device. Exercise-induced Δ[tHb] and Δ[Oxy] responses at each constant workload were evaluated as functional Δ[tHb] change (fΔ[tHb]) and functional oxygenation change (fΔ[Oxy]), respectively. Blood lactate concentration [La] was also evaluated after each exercise stage.

Results: At work rates 60%Wmax and below, after an initial decrease at the start of exercise, both Δ[tHb] and Δ[Oxy] showed progressive increases until the end of exercise. A significant positive correlation was found between fΔ[tHb] and fΔ[Oxy] (p<0.01). In addition, there was a significant negative relationship of [La] to fΔ[tHb] during exercise (p<0.05). These results provide evidence that increased muscle oxygenation during bicycle exercise up to 60%Wmax may be caused by increased O2 supply due to exercise-induced blood volume expansion. Subsequently, the cessation of increase in fΔ[tHb] at higher intensity exercise may lead to lower muscle tissue oxygenation and higher lactate accumulation.

key words: muscle oxygenation • muscle perfusion • oxygen uptake • lactate • bicycle exercise

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BACKGROUND

Muscle perfusion increases linearly in relation to work intensity, and peak muscle perfusion was reported to reach 50 to 100-fold higher than resting [1]. The increased muscle perfusion is caused by the result of interactions of central vascular control mechanisms, locally mediated vasodilation of resistance vessels, and the mechanical effects of muscle contraction [1]. However, it is reported that the local vascular control systems within the myogenic factors primarily cause exercise-induced functional hyperemia [2]. Metabolites from actively contracting muscle (i.e. adenosine, H+, lactic acid and CO₂) diffuse into the interstitial space to the resistance arterioles and cause vasodilation and capillary recruitment. Functional hyperemia may coordinate blood flow, red blood cell distribution, and decrease perfusion heterogeneity in skeletal muscle during exercise [3,4]. Furthermore, the increased muscle perfusion raises the O₂ supply to a level compatible with O₂ demand which may lead to less O₂ deficit [5].

Although exercise-induced functional hyperemia is one of the most important factors of O₂ supply to myocytes [6], there are only a few studies that have monitored functional hyperemia in humans because of the difficulty in detecting it. Some studies have monitored muscle perfusion during exercise using functional magnetic resonance imaging (fMRI) [7] and positron emission tomography (PET) [3,4]. However, the equipment is rather expensive and inconvenient for evaluation of muscle perfusion during dynamic whole body exercise.

Near infrared continuous wave spectroscopy (NIRcws) has been found to be a very useful technique for noninvasive measurement of tissue oxygenation and total hemoglobin (tHb) change [8,9]. The use of NIRcws is becoming more prevalent now in the field of work and exercise physiology [8–11]. NIRcws has a great advantage over invasive forms and other more expensive noninvasive forms because the NIRcws can monitor the changes in blood volume and oxygenation within specific regions of muscle during static contraction [11,12] and various forms of dynamic contraction [13–16]. As the body of research using the NIRcws technique builds, a better understanding of this technique’s reliability under specific conditions will be understood [9]. Since NIRcws can measure total hemoglobin change (Δ(tHb)) in muscle tissue during dynamic exercise, this technique has the potential to determine exercise-induced blood volume expansion noninvasively. The purpose of this study was to examine this Δ(tHb) response during bicycle exercise at various constant workloads using NIRcws in humans. We hypothesized that the Δ(tHb) during exercise may progressively increase as a result of dilation of the vascular bed and/or capillary recruitment at lower constant work rates.

MATERIAL AND METHODS

Subjects

Seven healthy volunteers (3 males, 4 females, age: 25.4 years, height: 170.7 cm, weight: 66.4±8.6 kg) participated in this study. All subjects were involved in various recreational activities, such as volleyball, soccer, jogging, and cycling, 2–3 times/wk, but none were trained athletes. All subjects were briefed about the experimental protocol, and written informed consent was obtained before the test. The Institutional Review Board of the University of Pennsylvania approved the research protocol.

Experimental protocol

Before the main experiment, all of the subjects performed a preliminary incremental bicycle exercise test (Monark 868, Sweden) to obtain their maximal workloads (Wmax). The male subjects started at 100 watts (W), and the female subjects started at 50 W. The workloads increased by 25 W every 2 min until exhaustion.

On a separate day, the subjects performed 5 min of bicycle exercise at 20, 40, 60, 80 and 100% Wmax. Each exercise stage was performed in random order and 25–30 min of recovery time intervened between trials. All of the bicycle tests were performed in an upright position, and the pedal frequency was maintained at 60 rpm during all exercise tests.

Pulmonary VO₂, HR and [La]

Pulmonary O₂ uptake (VO₂, in STPD), and carbon dioxide production (VCO₂, in STPD) were assessed using the breath-by-breath method with an online metabolic system (SensorMedics 2900, Yorba Linda, CA). The O₂ and CO₂ analyzers were calibrated before each experiment by utilizing gas mixtures of known composition. Heart rate was monitored continuously during the experiment by a 3-lead ECG (Biopack MP100 system, USA). Blood samples were taken via fingertip for blood lactate concentration ([La]) at 30 sec after each exercise stage, and analyzed by an enzymatic method (Accusport, Mannheim, Germany).

NIRcws

Changes in muscle oxygenation (Δ(Oxy)) and total Hb (Δ(tHb)) were measured by a two-wavelength (730 nm and 850 nm) light-emitting diode NIRcws (cwNIRS; NIM, Philadelphia, PA). The principle of the measurement and the specifications of the NIRcws have been fully described [8,9]. The level of oxygenation of hemoglobin and/or myoglobin (Hb/Mb) alters the absorption of the light in muscle tissue. For example, as Hb/Mb is oxygenated, the absorbance at 730 nm decreases and the absorbance at 850 nm increases, providing a different signal. The difference in signal (signal at 850 nm minus the signal at 730 nm) is defined as Δ(Oxy), which should be sensitive to Hb/Mb O₂ saturation and the sum signal to Δ(tHb) [9].

We used a NIRcws device developed to enable a high signal-to-noise ratio (S/N), such that the S/N of the present NIRcws device was 6.34-fold greater than the Runman oxymeter [17]. Data acquisition sampling frequency was set at 2 Hz. We used one source and four photodetectors to cover a 4.23 cm square area to only monitor a single muscle in this study. The source and detectors were installed in the center and in the corners of a square shaped probe, respectively. Each source-detector distance was 3.0 cm. The measurement probe was placed at a point one-third (1/3) from the patella to the greater trochanter. For reliable probe position, the distance was measured from the center of the patella and greater trochanter to the probe on the vastus lateralis mus...
The probe was firmly attached to the skin and no sliding was observed in any subject. Δ[tHb] and Δ[Oxy] were expressed in ΔμM, and were corrected for fat layer thickness effects by adapting the results of Monte Carlo simulation [19]. This calculation has its origin in our previous paper with Niwayama et al. [19], in which we calculated quantitative measurement of muscle oxygenation using NIRcwS with correction for the influence of fat layer thickness.

Statistics

The changes in recorded variables during the exercise experiments were analyzed by one-way ANOVA for repeated measurements. Following a significant $F$ test, pair-wise differences were identified using Tukey’s honestly significant difference (HSD) post hoc procedure. When appropriate, significant differences were also identified using Student’s paired t-test. Regression and correlation analyses were performed by the least squared residuals method. The level of significance was set at $p<0.05$.

RESULTS

[La] and Pulmonary VO$_2$

The [La] in relation to the percentage of pulmonary VO$_2$peak is displayed in Figure 1. [La] showed the classical curvilinear pattern of increase as a function of work rate. Baseline [La] was 1.7±0.1 mM, and the [La] did not significantly increase until 60%Wmax (3.0±0.2 mM), and increased more dramatically at higher work rates (80%Wmax; 5.3±0.4 mM, 100%Wmax; 8.2±1.0 mM).

Exercise-induced blood volume expansion

Typical responses of Δ[tHb] and Δ[Oxy] to bicycle exercise are illustrated in Figure 2. At the beginning of exercise, Δ[tHb] steeply decreased due to mechanical factors related to contraction. Following the steep decrease, Δ[tHb] reached minimum values within 15 sec after the start of exercise. Thereafter, as the exercise continued there was a progressive increase of Δ[tHb]. In this study, Δ[tHb] response at each work rate was evaluated from the minimum values occurring within 15 sec after the start of exercise (Δ[tHb]$_{\text{min}}$) to the maximal values occurring within 1 min before the cessation of exercise (Δ[tHb]$_{\text{max}}$), as functional blood volume change (ƒ-Δ[tHb]). In addition, Δ[Oxy] also decreased at the beginning of exercise due to the muscle pump squeezing blood and increased muscle O$_2$ consumption (muscle VO$_2$). After Δ[Oxy] reached minimum values within 1 min after the start of exercise, Δ[Oxy] also progressively increased as the exercise continued. Therefore, the Δ[Oxy] response at each work rate was evaluated from the minimum value occurring within 1 min after the start of exercise (Δ[Oxy]$_{\text{min}}$) to the maximal value occurring within 1 min before the cessation of exercise (Δ[Oxy]$_{\text{max}}$), as functional oxygenation change (ƒ-Δ[Oxy]), (Figure 2).

Relationship between ƒΔ[tHb] and [La]

Figure 3 shows the ƒΔ[tHb] during exercise in relation to the VO$_2$ peak. There were positive values of ƒΔ[tHb] at 20, 40 and 60%Wmax, and the peak value of the ƒΔ[tHb] (29.7±4.0 μM) was obtained at 40%Wmax. The positive value in the vertical axis indicates that Δ[tHb] increased as the exercise continued. In contrast, ƒΔ[tHb] at 80% and 100%Wmax only yielded a small change (10.1±5.0 μM) and a negative value (–13.6±5.0 μM), respectively. The ƒΔ[Oxy] at 80%Wmax was significantly lower than 40% and 60%Wmax (p<0.001), and the ƒΔ[Oxy] at 100%Wmax was significantly lower than 20%, 40% and 60%Wmax (P<0.001).

The ƒΔ[Oxy] in relation to %VO$_2$peak is displayed in Figure 4. As explained above, the positive value in the vertical axis indicates that the muscle tissue was oxygenating during exercise, not deoxygenating. There was a significant increase in ƒΔ[Oxy] at 20, 40 and 60%Wmax. ƒΔ[Oxy] at 80% and 100%Wmax produced a small change (4.0±6.2 μM) and a negative value (–43.2±9.3 μM), respectively. Similar to the
f-Δ[tHb], the f-Δ[Oxy] was significantly lower at 80%Wmax (p<0.05) and 100%Wmax (P<0.001) compared with 20, 40, and 60%Wmax. The relationship between f-Δ[tHb] and f-Δ[Oxy] was significantly correlated as shown in Figure 5. (p<0.01). In addition, there was a significant negative correlation of f-Δ[tHb] to [La] as shown in Figure 6 (p<0.05).

**Reproducibility of f-Δ[tHb] and f-Δ[Oxy]**

On a separate day, all of the subjects performed the same exercise protocol at 20, 40 and 60%Wmax to determine the reproducibility of the f-Δ[tHb] and f-Δ[Oxy]. The variation of coefficients for the f-Δ[tHb] and f-Δ[Oxy] were 6.3±2.4% and 7.2±2.6%, respectively.

**DISCUSSION**

The main results of this study are that after an initial drop in Δ[tHb] during the first minute of exercise, Δ[tHb] during exercise increased up to 60%Wmax, and this blood volume expansion was significantly correlated with f-Δ[Oxy] and [La]. These results demonstrate that the increased muscle oxygenation may be caused mainly by increased O2 supply due to arteriolar vasodilation and capillary recruitment at lower exercise intensities. Subsequently, no further increase in f-Δ[tHb] were seen at high exercise intensities, probably because of increased intramuscular pressure, which in turn may lead to lower muscle tissue oxygenation.

**Validity of f-Δ[tHb]**

As both Hb/Mb absorb light equally at the same wavelengths, it is quite difficult to separate the signal between Hb and Mb [9]. In this study, we used a NIRcws device which can measure concentration changes from the initial value at the beginning of the experiment. Total Mb concentration should not change during each exercise test, unless Mb leaked due to muscle damage. As the exercise type in the study was not eccentric contraction at the vastus lateralis muscle, it is suggested that the agonist muscle had less muscle damage and Mb leakage. In addition, all of the subjects completed the exercise tests within a day. This implies that Mb concentration in the skeletal muscle should not have changed during the experimental period. In this study, therefore, we pre-

**Figure 3.** f-Δ[tHb] during exercise in relation to the VO2 peak. The dotted line indicates the zero point of f-Δ[tHb] meaning Δ[tHb] did not change between the start and end of exercise. * P<0.05 and ** P<0.01 versus 20%Wmax; † P<0.05 and †† P<0.01 versus 40%Wmax, and ‡ P<0.05 and ‡‡ P<0.01 versus 60%Wmax.

**Figure 4.** f-Δ[Oxy] during exercise in relation to % VO2 peak. The dotted line indicates zero point of f-Δ[Oxy] meaning Δ[Oxy] did not change between the start and end of exercise. * P<0.05 and ** P<0.01 versus 20%Wmax; † P<0.05 and †† P<0.01 versus 40%Wmax, and ‡ P<0.05 and ‡‡ P<0.01 versus 60%Wmax.

**Figure 5.** Relationship between f-Δ[tHb] and f-Δ[Oxy] during exercise. Each dotted line indicates zero point of f-Δ[tHb] and f-Δ[Oxy], respectively. The solid bold line represents the linear regression line (r=0.975, p<0.01).

**Figure 6.** Relationship between f-Δ[tHb] and [La] during exercise. The dotted line indicates zero point of f-Δ[tHb]. The solid bold line represents the linear regression line (r=0.933, p<0.05).
sumed that the Δ[Hb] signal came only from Hb and was not contaminated by the Mb signal.

Moreover, most of the Hb signal is thought to come from small vessels because large arteries and veins have large Hb concentrations that absorb all the light [9]. It is unlikely that increased skin vasodilation affected the Δ[Hb] because Mancini et al [20] suggested that the interaction of skin vasodilation during exercise could be ignored. Therefore, the changes in NIRcws signals were mostly derived from the absorption of light in small vessels such as arterioles, capillaries and venules.

Contamination of Δ[Hb] measurement from the small vein

Upon the initiation of each exercise stage, Δ[Hb] and Δ[Oxy] decreased steeply due to blood redistribution such that this decreased Δ[Hb] may have been affected by the muscle pump squeezing blood through the capillaries and small vessels (Figure 2). Stick et al. [21] measured venous pressure at the ankle during bicycle exercise at 50W and found that when starting the exercise, venous pressure decreased within the first minute to a level which remained constant until the end of exercise. In this study, Δ[Hb] was defined as changing values of Δ[Hb] from the minimum values within 15 seconds after the start of exercise to the peak level later in the exercise bout. Therefore, it is suggested that after Δ[Hb] decreased as a result of mechanical factors related to muscle contractions at the start of exercise, venous blood volumes were constant during the bicycle exercise at 60%Wmax and below, the point at which [La] started to increase. In other words, any contamination effects of venules to the measurement of Δ[Hb] is thought to be negligible below the lactate threshold (LT). These results suggest that the increasing Δ[Hb] caused by exercise continuation below LT may be derived from exercise-induced functional hyperemia due to dilation of arterioles and/or capillary recruitment.

Δ[Hb] response

It has been reported that exercise-induced increases in blood flow to skeletal muscle are primarily the result of local vascular control systems within the muscle tissue [2]. In addition, the primary determinant of sustained exercise hyperemia in skeletal muscle is metabolic vasodilation [2]. This report suggests that metabolites from actively contracting muscles diffuse into the interstitial space to the resistance arterioles and cause vasodilatation and capillary recruitment demonstrated as Δ[Hb]. Because higher exercise intensity causes increased adenosine, phosphate, CO₂ and potassium, and decreased blood PO₂ and pH, arteriolar dilation may be increased by continued exercise [2]. However, at higher exercise intensities, O₂ supply to the muscle may be restricted by mechanical effects due to increased intramuscular pressure [1,22,23]. This implies that at higher exercise intensities the mismatch of O₂ supply with increased O₂ demand may lead to muscle tissue deoxygenation (Figure 5).

In this study, Δ[Hb] at 80% and 100%Wmax were below the values at lower work rates. The lower Δ[Hb] at higher work rates may be explained by increased intramuscular pressure. Stick et al. [21] found that at work rates 60%Wmax and below, venous blood volume remained steady during exercise after the initial drop of Δ[Hb] due to mechanical factors related to muscle contraction at the beginning of exercise. However, in our study, exercise at work rates higher than 80%Wmax induced greater lactate accumulation, and increased fast-twitch fiber recruitment. Subsequently, the greater fast-twitch fiber recruitment may have led to higher blood return from capillaries and/or venules. Therefore, we can not exclude the possibility that Δ[Hb] at 80% and 100%Wmax may have been influenced by the decreased blood volume from capillaries and/or venules during the bicycle exercise. We suggested that the progressive expansion in Δ[Hb] during continuous exercise below LT should be used for the purest evaluation of Δ[Hb], not the blunted response at higher work rates.

Relationships of Δ[Hb] to Δ[Oxy] and [La]

We found a strong negative relationship between Δ[Hb] and [La] (Figure 6). Hogan and Welch [24] reported that under hypoxic conditions, lactate production is increased even if muscle O₂ consumption is maintained. Recently, some studies reported a strong positive correlation between blood lactate level and epinephrine concentration which stimulated glycolysis via cAMP and relates to lactate production [24,25]. Richardson et al [26] suggested that lactate efflux was not related to intracellular PO₂, yet the lactate efflux was higher in hypoxia than normoxia. Furthermore, a strong relationship was found between net lactate efflux and arterial epinephrine concentration in hypoxia as well as normoxia. It is suggested that increased [La] during exercise may be influenced by elevated sympathetic drive more so in hypoxia. The combination of previous studies with our own present findings suggest that at higher exercise intensities, insufficient O₂ supply may cause muscle tissue deoxygenation, leading to increased sympathetic drive which may result in an increase in [La].

Conclusions

The Δ[Hb] responses during various constant workloads were measured as an indicator of exercise-induced functional hyperemia using NIRcws in humans. The Δ[Hb] increased during exercise below LT and this Δ[Hb] was significantly correlated with Δ[Oxy] and [La]. These results suggest that Δ[Hb] may be derived from dilation of cutaneous and/or from capillary recruitment. The Δ[Hb] could be used clinically to objectively evaluate changes in the function of microcirculation from peripheral vascular disease [27] and also from chronic endurance exercise training.

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