The relationship between muscle deoxygenation and activation in different muscles of the quadriceps during cycle ramp exercise

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Chin LM, Kowalchuk JM, Barstow TJ, Kondo N, Amano T, Shiojiri T, Koga S. The relationship between muscle deoxygenation and activation in different muscles of the quadriceps during cycle ramp exercise. J Appl Physiol 111: 1259-1265, 2011. First published July 28, 2011; doi:10.1152/japplphysiol.01216.2010.—The relationship between muscle deoxygenation and activation was examined in three different muscles of the quadriceps during cycling ramp exercise. Seven young male adults (24 ± 3 yr; mean \pm SD) pedaled at 60 rpm to exhaustion, with a work rate (WR) increase of 20 W/min. Pulmonary oxygen uptake was measured breath-by-breath, while muscle deoxygenation (HHb) and activity were measured by timeresolved near-infrared spectroscopy (NIRS) and surface electromyography (EMG), respectively, at the vastus lateralis (VL), rectus femoris (RF), and vastus medialis (VM). Muscle deoxygenation was corrected for adipose tissue thickness and normalized to the amplitude of the HHb response, while EMG signals were integrated (iEMG) and normalized to the maximum iEMG determined from maximal voluntary contractions. Muscle deoxygenation and activation were then plotted as a percentage of maximal work rate (%WR_{max}). The HHb response for all three muscle groups was fitted by a sigmoid function, which was determined as the best fitting model. The c/d parameter for the sigmoid fit (representing the %WRmax at 50% of the total amplitude of the HHb response) was similar between VL (47 \pm 12% WR_{max}) and VM (43 \pm 11% WR_{max}), yet greater (P < 0.05) for RF (65 \pm 13% WR_{max}), demonstrating a "right shift" of the HHb response compared with VL and VM. The iEMG also showed that muscle activation of the RF muscle was lower (P < 0.05) compared with VL and VM throughout the majority of the ramp exercise, which may explain the different HHb response in RF. Therefore, these data suggest that the sigmoid function can be used to model the HHb response in different muscles of the quadriceps; however, simultaneous measures of muscle activation are also needed for the HHb response to be properly interpreted during cycle ramp exercise.

incremental exercise; near-infrared spectroscopy; surface electro-myography

THE PHYSIOLOGICAL RESPONSE of humans to a ramp or incremental exercise test to exhaustion provides valuable information regarding the integration of the various physiological systems across exercise intensities. Using near-infrared spectroscopy (NIRS), Ferreira et al. (12) demonstrated that the increase in muscle deoxygenation (HHb) during cycle incremental exercise was sigmoidal in nature. The concentration changes of the NIRS-derived HHb signal is believed to be reflective of microvascular O₂ extraction (8, 13, 14, 21), assuming constant total Hb and that the proportion of the HHb signal originating from the microvasculature remains unchanged. Therefore, a sigmoidal profile of HHb suggests that the relationship between muscle microvascular blood flow (Q_m) and muscle O₂ utilization (Vo_{2m}) varies across the different exercise intensities (12) as follows: the shallow slope during light exercise suggests a faster increase in Q_m compared with Vo_{2m}, the steeper slope during moderate exercise reveals greater O2 extraction and hence a slower increase in Q_m relative to Vo_{2m} , and the plateau of HHb at the end of exhaustive exercise demonstrates a matching between the rate of increase in Q_m and Vo_{2m}. This sigmoidal profile of HHb has been shown in both trained and untrained individuals (4), during incremental step exercise (5), supine exercise (9), and in the distal and proximal regions of the vastus lateralis (VL) muscle (5).

However, many studies utilizing NIRS only measure from a single site (usually the VL muscle), which is viewed as the representative response of the whole leg muscle. Using multichannel NIRS system, it has been demonstrated that substantial variability exists for muscle deoxygenation (and thus the distribution of \dot{Q}_m relative to $\dot{V}o_{2m}$) within and between muscles of the quadriceps during constant cycling exercise (24, 25, 38, 42). As the relationship between muscle microvascular \dot{Q}_m and $\dot{V}o_{2m}$ varies across the range of exercise intensities during ramp exercise, a closer examination of HHb among different muscles within the leg is warranted. Boone et al. (5) recently showed that the sigmoid parameters of HHb were not different between the distal and proximal regions of VL; however, it remains to be determined whether a difference exists among various muscles of the quadriceps.

The information obtained from HHb provides insights into the relationship between \dot{Q}_m and $\dot{V}o_{2m}$; however, interpretation of HHb would also depend on the activation level of the muscle being examined. For instance, a rightward shift of the HHb sigmoid profile (relative to work rate) may indicate better perfusion and balance between \dot{Q}_m and $\dot{V}o_{2m}$; however, this may also be reflective of a reduced need for \dot{Q}_m and $\dot{V}o_{2m}$ that occurs with lower muscle activation. Phosphorescencequenching measurements of microvascular O_2 pressures ($P_{mv}O_2$) also indicate that the relationship between \dot{Q}_m and $\dot{V}o_{2m}$ following the onset of contractions in the rat muscle depend on the fiber type composition of the muscle (37). Muscles composed predominantly of slow-twitch fibers demonstrate faster adjustment of \dot{Q}_m relative to $\dot{V}o_{2m}$ and better

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matching of \dot{Q}_m and $\dot{V}o_{2m}$ compared with fast-twitch muscle fibers (3, 30). The difference in relationship between \dot{Q}_m and $\dot{V}o_{2m}$ that is inherent to the muscle fiber type could induce an attenuated increase in \dot{Q}_m across increasing work rates since incremental exercise is presumably characterized by a shift in recruitment of predominantly slow-twitch muscle fibers to more fast-twitch muscle fibers. In human muscles, substantial variations in muscle recruitment patterns and fiber type distribution within and across muscles would affect the relationship of \dot{Q}_m and $\dot{V}o_{2m}$ during cycling exercise (25), and further result in differences in the HHb profile between muscles of the quadriceps.

Therefore, the purpose of the present study was to determine the profile of muscle deoxygenation during cycle ramp exercise to exhaustion in three different muscles of the quadriceps [VL, rectus femoris (RF), and vastus medialis]. In addition, muscle activity was measured by surface electromyography (EMG) to examine the relationship between muscle deoxygenation and activation in these three muscle groups. It was hypothesized that 1) VL, RF, and VM would each demonstrate a sigmoidal profile of the HHb response, and that 2) any differences in the sigmoidal parameters among these muscle groups would be related to the level of muscle activation.

METHODS

Ethical Approval

This study was approved by the Human Subjects Committee of Kobe Design University, in accordance with the Declaration of Helsinki. Explanation of the experimental protocol, including any possible risks and benefits associated with the testing procedures, were discussed with the subjects, and written consent was obtained from all subjects before voluntary participation in this study.

Subjects

Seven young male subjects (age, 24 ± 3 yr; height, 170 ± 7 cm; weight, 61 ± 12 kg; mean \pm SD) participated in this study, and all were nonsmokers and free of known respiratory, cardiovascular, and metabolic disease.

Experimental Protocol

Subjects reported to the laboratory at least 2 h after their last meal, and having refrained from caffeine, alcohol, and strenuous exercise for 24 h before testing. All experimental protocols were carried out in a climate-controlled laboratory, where the ambient temperature and relative humidity were maintained at $\sim 22^{\circ}$ C and $\sim 50\%$, respectively.

Subjects performed the exercise tests on an electronically braked cycle ergometer (Combi 232C, Tokyo, Japan), in a seated upright position. The protocol began with a 2-min resting period, followed by 4 min of unloaded "baseline" exercise and the incremental exercise test. The ramp increase was 20 W/min, and subjects were instructed to maintain a pedal frequency of 60 rpm. The test was terminated when subjects were unable to maintain the target pedal frequency, despite verbal encouragement.

Measurements

*Pulmonary O*₂ *uptake (Vo*₂). Subjects breathed through a lowresistance hot-wire flowmeter for measurement of inspiratory and expiratory flows (Aeromonitor AE-300S; Minato Medical Science, Osaka, Japan). Calibration of the flowmeter was performed by inputting known volumes of room air at various mean flows and flow profiles. Respired gases obtained from samples drawn continuously from the mouthpiece were analyzed using a gas analyzer (a paramagnetic O_2 analyzer and an infrared CO_2 analyzer; Aeromonitor AE-300S; Minato Medical Science, Osaka, Japan). Precision-analyzed gas mixtures were used for calibration. Data collected every 13 ms were transferred to a computer, and gas volume and concentration signals were time-aligned by accounting for the delay in the sampling tube and the analyzer rise time relative to the volume signal. The algorithms of Beaver et al. (2) were used to calculate breath-by-breath alveolar gas exchange variables. Beat-by-beat heart rate was also monitored and recorded continuously by an electrocardiogram using a three-lead arrangement.

Muscle deoxygenation. Muscle deoxygenation (HHb) was measured simultaneously at three different sites using three separate channels from two time-resolved spectroscopy (TRS) NIRS (TRS-20 for two channels, Hamamatsu Photonics KK, Hamamatsu, Japan). This system is able to determine absolute concentration values of HHb as the distribution of in vivo optical path lengths are directly measured (43). The optodes were placed in an optically dense rubber holder that held the receiver and emitter in the same position, exactly 3 cm apart. Each assembly was placed on the muscle belly of three different muscles on the right leg: the VL, RF, and VM. All sites were shaved and wiped clean with alcohol before the optode assembly was attached to the leg by double-sided tape.

A detailed explanation of the principles of operation and algorithms utilized by the equipment is described elsewhere (34, 35). Briefly, the TRS consists of a picosecond light pulser that emits three wavelengths (760, 795, and 830 nm), with a frequency of 5 MHz and a full width at half maximum (FWHM) of 100 ps. The laser diodes and photomultiplier tube are connected to a lightweight plastic probe by optical fibers for single-photon detection. A time-correlated single-photon counting board (constant fraction discriminators, time-to-amplitude converters, A/D converters, and histogram memories) acquires timeresolved reflectance curves of the emerging photons, and estimation of the optical path length, reduced scattering coefficient (μ_s') and absorption coefficient (μ_a) is achieved by fitting the profile using diffusion theory (36). The output frequency was selected as 0.5 Hz. Muscle deoxygenation was determined using the measured μ_a (34, 35). Calibration of the equipment was performed before each test by measuring the instruments' responses when the input and receiving fibers face each other through a neutral-density filter in a black tube.

To account and normalize for the effect of subcutaneous adipose tissue thickness (ATT) on the amplitude of the HHb signal, subjects returned to the laboratory on a separate occasion for determination of their ATT and to perform an ischemic test at rest. Measurements of ATT were made using Doppler ultrasound (Logiq 400, GE-Yokogawa Medical Systems, Tokyo, Japan) at the same position as the optode sites over the VL, RF, and VM muscle. The influence of ATT on the NIRS signal was determined by examining the slope of the change in HHb during the ischemic test. Assuming resting Vo_{2m} was the same among all three muscle groups (refer to Methodological Considerations in DISCUSSION), differences in the slope of the change in HHb would likely be related to the overlying ATT. The NIRS optodes during the ischemic test were placed in the same position as in the incremental exercise test (guided by pen marks from the previous visit), over the VL, RF, and VM muscle. Subjects were seated in an upright position, with the occlusion cuff placed around the upper thigh of the right leg. Following 2 min of seated rest, the occlusion cuff was immediately inflated (E-20 Rapid Cuff Inflator, Hokanson, Bellevue, WA) to and held at 250 mmHg, until a plateau in the HHb signal (reflecting peak muscle deoxygenation) was observed.

Surface electromyography. Three separate bipolar electromyography (EMG) sensors were connected to a biometric data logger (BioLog DL-1000, S&ME, Tokyo, Japan) for continuous collection of EMG signals, with a sampling frequency of 1 kHz. Electrodes (Vitrode M, Nihon Kohden, Tokyo, Japan) were positioned just distal to the NIRS optode assembly on the VL, RF, and VM, and all sites were prepared by shaving, abrading, and cleaning the skin with alcohol. Before data collection, EMG signals were tested for movement

artifact and the EMG sensor body was then secured to the leg with surgical tape to help minimize any movement during exercise.

On a separate day, subjects performed three repetitions of maximal voluntary contractions (MVC) while seated upright on a chair with their knees at right angle to the ground. During the MVC, subjects were instructed to push their right leg "into" the floor, while an assistant restrained the subject from rising off the chair, thereby maximizing downward force production. As some subjects had difficulty producing acceptable values of MVC with this method, another three MVC were performed by having subjects extend and push their right leg against an immovable bar. All MVC were held for at least 5 s, and subjects were allowed to rest for at least 2 min before performing the next MVC. The position of the EMG electrodes was the same as in the incremental exercise test (which was outlined in the previous visit), and the skin was prepared as mentioned previously.

Data Analysis

Peak pulmonary Vo_2 was determined as an average of the last 30 s of the exercise ramp test. Analysis of the HHb signal was performed following correction for ATT. The slope of the change in HHb during the ischemic test reflects measurement sensitivity (33) and was determined from the start of occlusion to the point just before a plateau in the HHb was observed. The relationship between the HHb slopes and ATT for all muscle groups and all subjects (Fig. 1) was then fit with an exponential decay model as follows:

$$F(x) = y_0 + a \cdot e^{(-k \cdot x)} \tag{1}$$

where y_0 and *a* are constants and *k* is the rate of decay. The amplitude of the raw HHb signal was corrected by the predicted HHb slope value when ATT was 0 mm (i.e., value of the *y*-intercept).

The corrected HHb signal was normalized to the amplitude of the HHb response, which corresponded to the 60-s average of baseline HHb and the final 15 s of the ramp exercise test. The normalized HHb signal was interpolated into 1-s values, averaged by a 5-point moving average and plotted against both absolute work rate (in W) and percentage of the maximum work rate (%WR_{max}). This final profile was modeled with sigmoid (*Eq. 2*) and hyperbolic (*Eq. 3*) functions as follows (see Ref. 12):



Fig. 1. Determination of the correction value for muscle deoxygenation (HHb) by measurement sensitivity (the HHb slope during the ischemic test) and adipose tissue thickness (ATT). Data points from all subjects across muscle groups were fitted with a single 3-parameter exponential decay model. Data were corrected to an assumed ATT of zero.

$$F(x) = f_{o} + A / [1 + e^{-(-c+dx)}]$$
(2)

where f_0 is baseline, A is the total amplitude, c is a constant dependent on d, d is the slope of the sigmoid, and c/d gives the x value that corresponds to 50% of A; and

$$F(x) = ax/(b+x) \tag{3}$$

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where *a* is the asymptotic value and *b* is the *x* value that corresponds to 50% of the total amplitude. Determination of the best fit function for the HHb profile was by visual examination and the "goodness of fit" (i.e., R^2 value). In addition, the Akaike Information Criterion (AIC) was used to determine the function that provided a superior fit for the HHb response, as used previously by Boone et al. (5) and DiMenna et al. (9). As the sigmoid function was the best fit for all subjects across muscle groups, only results for the sigmoid function are presented.

Raw EMG signals were rectified, band-pass-filtered, and integrated (m-Scope, S&ME, Tokyo, Japan). The integrated EMG (iEMG) signals were averaged into 30-s bins (i.e., every 10 W) and normalized to the maximum iEMG from the MVC trials. As iEMG values from the knee extension MVC trials were greater and more consistent than the MVC trials performed with the leg bent at a 90° angle, maximum iEMG was determined as the average of the three highest 1-s iEMG values from the knee extension MVC trials.

Statistical Analysis

Parameters for the sigmoid function of HHb were analyzed using a one-way analysis of variance (ANOVA) for repeated measures. Comparisons for all variables across work rate were analyzed by two-way ANOVA for repeated measures with the main effects of muscle group and percent work rate. A significant *F*-ratio was analyzed using Tukey's post hoc analysis with statistical significance accepted at P < 0.05. All values are expressed as means \pm SD.

RESULTS

Subjects achieved a mean peak $\dot{V}o_2$ of 48 ± 8 ml·kg⁻¹·min⁻¹ and a maximum work rate of 288 ± 43 W, with the incremental test lasting an average of 865 ± 129 s.

Correction of HHb Amplitude by ATT

The relationship between the HHb slopes (obtained from the ischemic test) and ATT for all subjects and muscle groups was fit with a three-parameter exponential decay function ($R^2 = 0.74$) as shown in Fig. 1. The HHb amplitude was then corrected by the slopes determined from the ischemic test, and normalized to an ATT of 0 mm.

HHb Response

The sigmoid parameters of the HHb response across normalized WR for VL, RF, and VM are presented in Table 1, and the sigmoid fits for the group mean normalized HHb values are represented in 1% (Fig. 2A) and 5% WR increments (Fig. 2B) across normalized WR. The sigmoid function provided a reasonable fit for all muscle groups, as demonstrated by the coefficient of determination (R^2 : VL, 0.87; RF, 0.80; VM, 0.91) and results from the AIC comparison. One subject showed a HHb response that was neither sigmoid nor hyperbolic for all muscle groups, so these data were excluded from further NIRS analysis. Baseline HHb values (f_o) for the sigmoid function were higher (P < 0.05) in RF compared with VM, while VL was not different from the other two muscle groups. The amplitude of the HHb response was smaller (P <

	fo, %WR _{max}	A, %WR _{max}	c/d		d		
			W	%WR _{max}	W	%WR _{max}	R^2
VL RF VM	-5 ± 9^{ab} 4 ± 12^{a} -15 ± 8^{b}	127 ± 11^{a} 99 ± 19 ^b 104 ± 13 ^{ab}	139 ± 41^{a} 184 ± 40^{b} 123 ± 40^{a}	47 ± 12^{a} 65 ± 13^{b} 43 ± 11^{a}	$\begin{array}{c} 0.02 \pm 0.01^{a} \\ 0.03 \pm 0.01^{a} \\ 0.02 \pm 0.01^{a} \end{array}$	$\begin{array}{c} 0.05 \pm 0.03^{\rm a} \\ 0.09 \pm 0.04^{\rm a} \\ 0.05 \pm 0.01^{\rm a} \end{array}$	0.87 0.80 0.91

 Table 1. Parameters for normalized HHb corrected by ATT, fit with a sigmoid function during cycle ramp exercise for 3 different muscle groups

Values are means \pm SD. ATT, adipose tissue thickness; VL, vastus lateralis; RF, rectus femoris; VM, vastus medialis; WR_{max}, maximal work rate; f_o , baseline muscle deoxygenation (HHb); A, amplitude; c/d, x-value at 50% of A; d, slope of sigmoid. Significant differences (P < 0.05) between muscles are denoted by different symbols (i.e., a, b, c); similar symbols demonstrate no differences.

0.05) in RF vs. VL, but no difference was observed compared with VM. The c/d parameter of the sigmoid function (expressed as either absolute WR or %WR_{max}) was greater (P < 0.05) for RF than for VL and VM. Therefore, the HHb response for RF was "right-shifted" compared with VL and



Fig. 2. Group mean and sigmoidal fit of the normalized mean HHb response for vastus lateralis (VL), rectus femoris (RF), and vastus medialis (VM) across normalized work rate (WR) represented in 1% (A) and 5% (B) WR increments. *Significantly different (P < 0.05) from VL and VM. †Significantly different (P < 0.05) from VL. ‡Significantly different (P < 0.05) from VL.

VM (Fig. 2*A*), and associated with a higher WR at 50% of the total HHb amplitude. No difference was observed in the slope (*d*) of the HHb response amongst muscle groups. Specific comparison of the muscle groups across percent of maximum WR revealed no difference at the beginning and end of the incremental exercise test; however, normalized HHb for RF was lower (P < 0.05) than VL and/or VM between 35 and 80% of WR_{max} (Fig. 2*B*).

iEMG Response

The mean normalized iEMG response for VL, RF, and VM is shown in Fig. 3 as a function of normalized WR. The mean iEMG values elicited for MVC was 76 ± 14 mV for VL, 50 ± 25 mV for RF, and 102 ± 26 mV for VM. While no difference was observed among muscle groups at 5% WR_{max}, the smaller increase in iEMG for RF resulted in lower (P < 0.05) muscle activation in RF vs. VL and VM throughout the remainder of the ramp exercise. The increase in iEMG was similar between VL and VM for the majority of the incremental test, where only at maximal exercise was the activation of VM lower (P < 0.05) than VL.

Muscle Deoxygenation and Muscle Activation

The ratio of the percent change from baseline of muscle deoxygenation relative to muscle activation $[(\%\Delta HHb)/(\%\Delta iEMG)]$ across $\%WR_{max}$ is presented in Fig. 4. The ratio of $[(\%\Delta HHb)/(\%\Delta iEMG)]$ was higher in RF than VL and VM at 10% WR_{max}, however was not different from VL and VM throughout the remainder of the incremental exercise test. No difference was observed between VL and VM.



Fig. 3. Mean normalized integrated EMG (iEMG) response for VL, RF, and VM across normalized WR. Data shown are means, with SE bars shown only at 100% maximal WR (%WR_{max}) for clarity. *Significantly different (P < 0.05) from VL and VM. †Significantly different (P < 0.05) from VL.



Fig. 4. Ratio of the percent change of HHb relative to iEMG from baseline for VL, RF, and VM across normalized WR. Data shown are means, with SE bars shown only at 100% WR_{max} for clarity. *Significantly different (P < 0.05) from VL and VM.

DISCUSSION

To our knowledge, this is the first investigation that examined the absolute microvascular oxygenation in conjunction with muscle activation in different muscles of the quadriceps during cycling ramp exercise. Previous studies have focused mainly on the VL, and little is known about the HHb responses of other muscle groups within the quadriceps (RF and VM). In addition, the level of muscle activation would also affect the HHb response, yet concurrent measurements of muscle activation and muscle deoxygenation during ramp incremental exercise have not been fully investigated. The aim of the present study was to examine the relationship between muscle deoxygenation and activation during ramp incremental cycle exercise to the limit of tolerance in different muscles of the quadriceps. We measured muscle deoxygenation by TRS-NIRS, muscle activation by iEMG, and adiposity at each site to account for the influence of ATT on absolute HHb values. The main findings in this study were that 1) all three muscles studied (VL, RF, and VM) displayed HHb responses that were appropriately modeled by sigmoid functions; 2) while the responses of HHb for VL and VM were similar, the RF muscle showed a "right-shifted" HHb response such that deoxygenation was lower in RF between 35 and 80% WR_{max} compared with VL and VM; 3) the relative iEMG for RF was lower than VL and VM throughout the ramp exercise test; and 4) the ratio of the percent change of HHb relative to iEMG from baseline was not different among muscle groups between 15 and 100% of WR_{max}, demonstrating the sigmoid increase of HHb was related to the increase in muscle activation for all muscle groups.

In agreement with previous studies, VL displayed a sigmoid curve of the HHb response during cycle ramp exercise to exhaustion (4–6, 9, 12). However, a novel finding in the present study was that other muscles of the quadriceps (the RF and VM) also displayed a sigmoid HHb response. All subjects showed a high R^2 value (≥ 0.85) for the sigmoid fit of the HHb response in VM, and profiles were very similar to that of VL. For RF, although the sigmoid function adequately modeled the HHb response (mean $R^2 = 0.80$), R^2 values were generally lower than VL and VM and were not consistent across all subjects. Therefore, while the HHb response for all three muscles of the quadriceps can be modeled by a sigmoid function, a lack of consistency with the RF muscle may suggest a different HHb response in certain subjects in this muscle group.

Comparisons of the parameters for the sigmoid function revealed differences among the muscle groups for matching Q_m and Vo_{2m} during various stages of ramp exercise. While VL and VM showed similar HHb responses throughout incremental exercise, the RF muscle demonstrated a HHb response that was shifted to the right suggesting that for a given percent of maximum work rate, O2 extraction was lower in the RF muscle compared with VL and VM. Examination of the normalized HHb profiles revealed a lower percent HHb in RF compared with VL and VM between 35 and 80% of $WR_{\rm max}$ (Fig. 2B). A "right-shift" of the HHb response may suggest a better matching of the increase in \dot{Q}_m and $\dot{V}_{O_{2m}}$ at a given work rate, as seen in individuals with high aerobic fitness, such as reported by Boone et al. (4) for trained cyclists compared with physically active students. A strong correlation was also observed between c/d and indexes for aerobic fitness (4), and it was suggested that the higher oxidative capacity in the cyclists, and perhaps the higher percentage of type 1 muscle fibers, were responsible for the right shift in the HHb response. In the present study, VL, RF, and VM were compared within the same individual; therefore difference in aerobic fitness was not a factor. It also appears unlikely that inherent differences in percentage of muscle fiber type in VL, RF, and VM are responsible for the HHb response. While VL reportedly consists of a greater proportion of type 2 muscle fibers compared with VM (49), no difference was observed in the HHb response between VL and VM. In addition, a lower percentage of type 1 muscle fibers in RF compared with VM (20) should theoretically shift the sigmoid HHb profile of RF to the left (rather than the right). Therefore, the right shift of HHb in RF in the present study is not likely a result of differences in training status, nor of different fiber type characteristics, among the quadriceps muscles within the same subject.

The availability of EMG data, however, suggests that the HHb response observed in RF is related to the pattern of muscle activation. The normalized iEMG signal was consistently lower in RF compared with VL and VM, suggesting lower muscle activation in RF throughout ramp exercise, and therefore a reduced requirement for O₂ extraction. While previous studies have measured EMG activity in two or more muscles of the quadriceps during incremental ramp cycling exercise (19, 26, 27, 46, 48), patterns of muscle activity between muscle groups were not compared. During constant cycling exercise at various intensities, studies utilizing EMG measures and/or the transverse relaxation time (T2) of magnetic resonance images (MRI) have demonstrated either lower (10, 11, 40, 45) or similar (16, 19, 40, 41) activation of RF compared with VL and/or VM. Perhaps the different findings within the literature are a result of variations in cycling conditions, exercise protocol, and/or subjects examined among the studies. Indeed, saddle height (15, 22), positioning of the foot on the pedal (11), use of toe-clips (11, 31), pedaling rate (11, 28, 29, 32, 44), subject fitness (19, 40), and cycling experience (19, 47) have all been shown to affect muscle activation within the quadriceps.

In the present study, the lower muscle activation in RF vs. VL and VM may be related to the function of these muscles during cycling. VL and VM are monoarticular muscles that cross only the knee joint and act to extend the knee. These vasti

muscles function as work generators (51) and are two of the most active muscles within the quadriceps during cycling exercise (11). Indeed, VL and VM are highly active (11, 22, 32) during the phase when pedal forces are greatest (7, 17). In contrast, RF is a biarticular muscle that crosses the hip and knee joints to flex the hip and extend the knee. However, during a pedal revolution, simultaneous flexion and extension is occurring at the hip and knee, creating a paradox for the RF muscle (1, 15). Examination of the functional role of biarticular muscles during cycling reveals that biarticular muscles support monoarticular muscles by transferring muscle power to the joints to achieve the most efficient mechanical output (39, 50, 51). Also, the RF muscle contributes to the smooth rotation of the crank arm by providing forward or backward force when the pedal is at the top or bottom dead center, respectively (18, 39). Therefore, monoarticular and biarticular muscles serve different roles in multijoint movements like cycling, which would explain the lower iEMG response observed in RF compared with VL and VM in the present study.

To examine the relationship between muscle activity and O_2 extraction, the ratio of the percent change from baseline for HHb and muscle activation was examined (Fig. 4). Except for an early difference between RF and the vasti muscles at 10% WR_{max}, all three muscle groups showed a consistent and similar response across increasing WR, demonstrating a close association between muscle deoxygenation and activation throughout the incremental exercise test. This proportional increase in O₂ extraction that accompanies muscle activation supports our hypothesis that the rightward shift of HHb in RF was related to lower muscle activation. The difference in ratio of the percent change of muscle deoxygenation relative to activation at 10% WR_{max} (range of 24–36 W for subjects) may be related to the difficulty at controlling the speed of the flywheel at low workloads, early on during incremental exercise. Mohr et al. (31) found that interpretation of EMG data during 0 and 300 kg/min of cycling at 60 rpm was not possible due to low-amplitude signals, while subjects had trouble controlling their speed with a light flywheel. Therefore at low resistance, subjects may have a less consistent pedal rate, resulting in variable patterns of muscle activation, in an effort to refrain from pedaling faster than 60 rpm.

In the present study, the sigmoidal HHb response in the presence of a linear increase in muscle activation during incremental exercise to fatigue demonstrates that the increase in HHb within the quadriceps muscle is not related to the increase in muscle activation per se, but rather is consequent to the balance between O₂ delivery and O₂ utilization within the recruited muscle. The shallow slope of HHb in the VL and VM may be reflective of early recruitment of muscle fibers (presumably slow-twitch type 1) that have better matching and maintenance of Q_m and Vo_{2m} than fibers recruited later that show poor capillarity and potential for flow increases. In contrast, the rightward shift of the HHb response for RF relative to VL and VM is likely due primarily to very low activation of RF (<5% of normalized iEMG) up to 40% WR_{max}. As work rate continues to increase during incremental exercise, the steeper slope of HHb in each of the muscles may represent a nonlinear Q_m and Vo_{2m} relationship inherent to muscle fiber types that results in a slower increase in \dot{Q}_m as muscle fiber recruitment patterns shift from predominantly slow-twitch muscle fibers to include more fast-twitch muscle fibers (12). Therefore, the rightward shift in RF may demonstrate a "delayed" activation/recruitment of fast-twitch muscle fibers that, once recruited, respond similarly to those in VL and VM following activation.

Methodological Considerations

In the present study, to examine the influence of ATT on muscle deoxygenation, resting $\dot{V}o_{2m}$ during cuff occlusion was assumed to be similar among the muscle groups of the quadriceps within a subject. Although heterogeneity of the $\dot{V}o_{2m}$ to \dot{Q}_m ratio in muscles of the quadriceps was previously demonstrated using positron emission tomography (PET) (23), no significant difference in $\dot{V}o_{2m}$ was observed between the different muscle regions at rest (Figure 2 of Ref. 23, and personal communication between Dr. Koga and Drs. Kalliokoski and Laaksonen). Therefore, it is currently unknown whether the degree of heterogeneity for resting $\dot{V}o_{2m}$, if any, would affect the increase of muscle deoxygenation during thigh occlusion. Further studies are warranted to develop better correction methods for removing the influence of ATT on the NIRS signal within and across subjects.

Conclusion

This study demonstrated that in different muscles of the quadriceps (VL, RF, and VM), the profiles of muscle deoxygenation during cycle ramp exercise are adequately described by a sigmoid function. However, differences existed in muscle deoxygenation between muscle groups, where a rightward shift of HHb was observed in the RF muscle compared with VL and VM. Concurrent measurement of muscle activation by surface electromyography revealed that this HHb response of RF was related to lower muscle activation during incremental exercise.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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