Inflection points of cardiovascular responses and oxygenation are correlated in the distal but not the proximal portions of muscle during incremental exercise

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EXERCISE STIMULATES THE CARDIOVASCULAR response by two neural mechanisms: central command (3) and the exercise pressor reflex (17, 32). The exercise pressor reflex is a feedback mechanism that acts via afferent nerves, which arise from the peripheral system and are capable of sensing chemical and mechanical stimuli (13). One such chemical stimulus is a metabolic error signal resulting from the mismatch between metabolic demand and oxygen supply in exercising muscle (6).

Oxygen supply is heterogeneous in the skeletal muscle of animals (15, 21) and humans (11, 18) at rest and during exercise. This heterogeneity in blood supply between proximal and distal portions (18, 21) within a given muscle is remarkable. Within a given muscle, intramuscular pressure is greater in the distal portion than in the proximal portion.

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determined in the right leg with use of the Cybex isokinetic dynamometer (Lumex, Ronkonkoma, NY). The average of three attempts was taken as the subject’s maximal torque.

We used the modified incremental exercise test originally reported by Kagaya (10). Exercise consisted of incremental 30-s static knee extension exercises, each separated by 30 s of recovery in a supine position. The initial load was 5% of maximal voluntary contraction (MVC), and the load was increased by 5% MVC every 60 s until exhaustion. Subjects performed incremental static knee extensions to an average of 59.3% of MVC (SD 7.9%, range 55–75%). Subjects monitored their exerted torque with an oscilloscope (DCS7020, Kenwood, Tokyo, Japan).

Measurements. Heart rate (HR) was determined by use of standard ECG leads (OEC-8108, Nihon Kohden, Tokyo, Japan). Arterial blood pressure was measured with a finger cuff (2300 Finapres, Ohmeda, Englewood, CO). The monitoring finger cuff was placed around the middle finger of the left hand. Averaged values were calculated from HR and mean arterial blood pressure (MAP) values obtained during the final 5 s of each 30-s exercise interval.

Calf blood flow in the nonexercising leg was measured by venous occlusion plethysmography using a mercury-in-Silastic strain gauge (EC-5R, Hokanson, Bellevue, WA). The left leg was maintained in a horizontal position at heart level. The strain gauge was placed around the largest area of the left calf. A pneumatic cuff placed around the thigh was inflated to 60 mmHg to measure calf blood flow during the final 5 s of each 30-s exercise interval. As an indicator of sympathetic nerve activity (7, 22, 25, 26), calf vascular resistance (CVR) in the nonexercising leg was calculated by dividing MAP by calf blood flow.

To provide an indicator of muscle oxygenation, the change in optical density (OD) of oxygenated hemoglobin (O2Hb) was measured at 1 Hz by using a commercially available device (OM-200; Shimadzu, Kyoto, Japan) in near-infrared continuous-wave spectroscopy mode. One NIRS probe was placed on the proximal portion and one NIRS probe on the distal portion of the vastus lateralis muscle; the
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portions of the muscle were determined by measuring 25% (proximal) and 75% (distal) of the length between the greater trochanter and the knee joint in the exercising leg. The light emitter-detector distance of the device was 40 mm. This apparatus uses three wavelengths of near-infrared light to measure changes in OD in O$_2$Hb and deoxygenated hemoglobin (HHb). O$_2$Hb and HHb were calculated according to the following equations:

$$\Delta[O_2Hb] = -1.631 \cdot \Delta \text{Abs}_{780} + 0.683 \cdot \Delta \text{Abs}_{805} + 1.605 \cdot \Delta \text{Abs}_{830}$$

$$\Delta[Hb] = 1.970 \cdot \Delta \text{Abs}_{780} - 0.314 \cdot \Delta \text{Abs}_{805} - 1.195 \cdot \Delta \text{Abs}_{830}$$

where $\Delta \text{Abs}_{780}$, $\Delta \text{Abs}_{805}$, and $\Delta \text{Abs}_{830}$ are the absorbance changes at the near-infrared light wavelengths of 780, 805, and 830 nm, respectively. The equations had been experimentally determined by the manufacturer. The changes in OD for total hemoglobin (total Hb) were calculated by summing the changes in OD of O$_2$Hb and HHb. $\Delta[O_2Hb]$ and $\Delta[Hb]$ (where $\Delta$ represents change and brackets denote concentration) were calculated with respect to an initial arbitrarily set value equal to zero and expressed in arbitrary units. The sum of the two variables, $\Delta[O_2Hb + HHb]$, reflects changes in the “total Hb volume” in the muscle region of interest, whereas the difference between the two variables, $\Delta[O_2Hb - HHb]$, reflects an “oxygenation index.” Average $\Delta[O_2Hb]$, $\Delta[Hb]$, $\Delta[O_2Hb + HHb]$, and $\Delta[O_2Hb - HHb]$ values were calculated during the final 5 s of each 30-s exercise interval.

Myoelectric activity was detected by surface electromyography (EMG) and recorded by use of bipolar 5-mm-diameter Ag–AgCl electrodes with an interelectrode distance of 40 mm. The EMG electrodes were placed on the same muscle portions used to measure oxygenation. Signals were amplified by a bioelectric amplifier (AB-621G; Nihon Kohden), and the EMG for each muscular contraction was integrated by using an EMG integrator (MacLab; ADInstruments, Castle Hill, Australia). Maximum integrated EMG (iEMG) was calculated during the maximum knee extension test before the exercise session. The iEMG value of each contraction was normalized to the maximum value. Averaged iEMG values were calculated during the final 5 s of each 30-s exercise interval.

**Calculation of inflection points.** Inflection points for MAP, CVR, and $\Delta[O_2Hb - HHb]$ were determined by iteratively fitting different combinations of two linear regressions to contiguous experimental points obtained during incremental exercise and by evaluating which combination yielded the lowest sum of squared residuals (5). This analysis did not use resting values for each variable. Although the number of averaged beats differed by five to eight beats at each workload, we calculated average values of MAP and HR in the final 5 s of each workload to match sampling time with other parameters such as NIRS, calf blood flow, and iEMG.

On a day other than the day of the exercise session, an ultrasonic apparatus (SSD-1000, Aloka, Tokyo, Japan) was used to measure thigh fat thickness in the same muscle portions used to measure oxygenation. Thigh fat thickness did not differ significantly between proximal ($4.0 \pm 0.4$ mm) and distal ($3.4 \pm 0.4$ mm) portions (means $\pm$ SE).

**Statistical analysis.** All data are represented as means $\pm$ SE. For regional parameters, a two-way ANOVA, with load and portions as main effects, was used to determine significant differences. If the $F$-test was significant, pairwise comparisons were performed by using Scheffé’s post hoc test. The Student’s unpaired $t$-test was used to test differences between inflection points of oxygenation in the proximal and distal portions of the vastus lateralis muscle. Simple linear regression analysis was used to determine the relationship between the inflection points of cardiovascular responses and muscle oxygenation. Values of $P < 0.05$ were considered significant.

**RESULTS**

Figure 1 summarizes the changes in cardiovascular and regional variables. HR, MAP, and CVR significantly increased with increasing workload (Fig. 1, A–C). $\Delta[O_2Hb - HHb]$ and iEMG changed significantly with increasing workload (Fig. 1, E and F). In contrast, $\Delta[O_2Hb + HHb]$ did not change significantly with increasing workload (Fig. 1D). There were significant differences between muscle portions in $\Delta[O_2Hb + HHb]$ and $\Delta[O_2Hb - HHb]$ but not in normalized iEMG; no significant interactions were observed for any variable.

Figure 2 shows individual data and the group mean for variables MAP, CVR, $\Delta[O_2Hb + HHb]$, and $\Delta[O_2Hb - HHb]$. Inflection points of these variables were identified by calculating the intersection of two linear regression equations; in these figures, the solid and dotted lines represent the regression fits of the shallow and steeper slopes that occur across a range of workloads. One subject (subject 2) showed a leveling off in $\Delta[O_2Hb - HHb]$ in the proximal portion at the highest exercise workload. This value was not included in the regression analysis. We were unable to measure calf blood flow and to obtained data for CVR at 55% MVC one subject (subject 4); however, the inflection point of CVR was clearly demonstrated. All variables exhibited similar patterns of change, except for $\Delta[O_2Hb - HHb]$ in subject 4. The inflection points of each parameter are clearly evident in both the individual and mean responses.

Figure 3 shows the values for the inflection point of $\Delta[O_2Hb - HHb]$ in both proximal and distal portions during incremental exercise. The inflection point in the proximal portion was significantly lower than that in the distal portion (28.5 ± 3.0 vs. 39.5 ± 3.0% MVC, respectively, $P < 0.05$); that is, the inflection point occurred at a lower percentage of MVC in the proximal portion than in the distal portion. In addition, in the distal portion the inflection points for MAP and CVR occurred at similar percentages of MVC (MAP, 41.2 ± 2.4% MVC; CVR, 39.8 ± 3.1% MVC).

Figure 4 shows the relationship between the inflection points of cardiovascular variables and $\Delta[O_2Hb - HHb]$. The inflection point of MAP was significantly correlated with that of $\Delta[O_2Hb - HHb]$ in the distal portion ($r = 0.89, P < 0.01$) but not in the proximal portion ($r = 0.05, not significant$). Similarly, the inflection point of CVR was significantly correlated...
with that of $\Delta [O_2Hb - HHb]$ in the distal portion ($r = 0.89$, $P < 0.05$) but not in the proximal portion ($r = 0.07$, not significant).

**DISCUSSION**

The two main purposes of this study were to quantify regional differences in the inflection point of muscle oxygenation and to clarify their relationship with systemic cardiovascular responses.

**Physiological factors that might have affected the NIRS signal.** NIRS is used to monitor oxygenation status in exercising muscles (5, 19, 20). The subcutaneous fat layer can strongly influence the NIRS signal (16). A previous review by McCully and Hamaoka (16) showed that in NIRS the light travels in a shallow arc to a penetration depth of about one-half the separation distance into the tissue. Thus, because the light emitter-detector distance of the device used in the present study was 40 mm, we estimate that the penetration depth was $\sim 20$ mm. The thickness of adipose tissue where NIRS measurements were performed did not differ significantly between portions ($4.0 \pm 0.4$ mm in the proximal and $3.4 \pm 0.4$ mm in the distal portions; see MATERIALS AND METHODS). Thus the NIRS signal reflected the metabolic changes occurring mainly in the muscle tissue in both muscle portions. Similarly, the fat layer would not have affected the inflection point of the oxygenation index.

**Regional differences in changes in total Hb volume and oxygenation between portions.** Because the normalized iEMG did not differ between portions during incremental exercise (Fig. 1F), we expected that muscular electrical activity would also be similar in the two portions. However, deoxygenation during incremental exercise was greater in the distal portion than in the proximal portion (Fig. 1E). This result confirms data from previous studies by Miura and colleagues (19, 20), who showed that regional differences in oxygenation status are consistent with regional differences in muscle architecture, which are related to regional differences in intramuscular pressure. Ameredes and Provenzano (1) showed that intramuscular pressure is greater in the distal portion than in the proximal portion of a muscle. Higher intramuscular pressure would inhibit circulation during muscle contraction, which might affect the inflection point of muscle deoxygenation. An alternative explanation is that, within a single muscle, the proximal portion contains a higher percentage of slow oxidative fibers than does the distal portion (30, 33). Oxidative fibers have a higher aerobic capacity and capillary density (15), so that the proximal portion might have a higher aerobic capacity and capillary density than the distal portion. It is possible that during incremental exercise deoxygenation occurs to a lesser extent in the proximal portion because of the higher blood flow and oxygen supply to this portion than in the distal portion.

The total Hb volume in the proximal portion was higher than in the distal portion during exercise; this finding supports the data from Miura and coworkers (19, 20). However, we observed no significant changes with increasing workload in both muscle portions (Fig. 1D). Although this result contrasts with data from previous studies (19, 20), this discrepancy can be explained by differences in body position (e.g., standing vs. supine) or the type of muscle contraction (e.g., dynamic vs. static) between our study and previous studies (19, 20). During static muscle contraction, as used in our study, intramuscular pressure inhibits arterial inflow to and venous outflow from the exercising muscle preventing any change in total Hb volume. In contrast, rhythmic muscle contraction as used by Miura and colleagues elicits arterial backflow and increases venous blood flow via the muscle pump; consequently, total Hb volume decreased in both portions of the exercising muscle. Further-
more, hydrostatic pressure and sympathetic nerve activity, which are greater in standing than in supine position, might have promoted a decrease in total Hb volume in the previous studies (19, 20).

Relationships between the inflection points of cardiovascular variables and muscle oxygenation. The inflection point of the oxygenation index was tightly coupled to that of cardiovascular variables in the distal portion but not in the proximal portion (Fig. 4). The exercise pressor reflex is a feedback mechanism that acts via afferent nerves arising from the peripheral system, which are capable of sensing chemical and mechanical stimuli. Intramuscular pressure increases linearly with increasing exerted torque during isometric muscle contraction (2, 27). In our study, muscle mechanical stimuli might have increased in both portions independent of the inflection point. In contrast, one chemical stimulus is a metabolic error signal resulting from the mismatch between metabolic demand and oxygen supply in exercising muscle (6). Evaluation of the muscle oxygenation index by NIRS reflects the balance between oxygen utilization and oxygen delivery. Grassi et al. (5) showed that the inflection point of muscle deoxygenation is coupled with that of blood lactate accumulation. During muscle contraction, sympathetic nerve discharge evoked by the exercise pressor reflex is associated with the accumulation of lactic acid (4, 12) and a decrease in muscle pH (28, 29, 31), which were enhanced by muscle anaerobic metabolism.

As mentioned above, one possible explanation for regional differences in sensing the exercise pressor reflex might relate to differences in the longitudinal distribution of skeletal muscle fiber type between proximal and distal portions (30, 33). Wilson et al. (34) showed that the fiber type of the contracting muscle influences the magnitude of the pressor response to static contraction; that is, static muscle contractions elicit a larger pressor reflex in predominately glycolytic muscle than in primarily oxidative muscle. An alternative explanation is that the distribution or number of afferent nerves may differ between proximal and distal portions within a muscle. Kumazawa and Mizumura (14) reported that muscular polymodal receptors are more frequently found in the head, the tail, and the edge of dog muscle. Further neurophysiological investigation is needed to identify the distribution of afferent nerves in human muscle.

The exercise pressor reflex is affected by muscle mass (9) and is greater when arising from forelimb as opposed to hindlimb muscles (8, 24). To date, it is unclear whether there are regional differences in the exercise pressor reflex within an exercising muscle. However, our present data suggest that the metabolic error signal resulting from the mismatch between metabolic demand and oxygen supply in exercising muscle is not homogenous, and consequently the exercise pressor reflex is not uniform within an exercising muscle.

Regional differences in the inflection point of the muscle oxygenation index within a given muscle. As mentioned above, several factors such as intramuscular pressure (1), muscle architecture (20), and muscle fiber type (34) may explain regional differences in muscle oxygenation. Considering data from previous reports, we hypothesize that the inflection point of muscle oxygenation would occur in the distal portion before the proximal portion. Surprisingly, our data indicated the opposite, that the inflection point occurred earlier in the proximal portion (Figs. 2 and 3). A possible explanation is that an additional inflection point in the proximal portion might appear at higher workload (>80% MVC). We speculate that the inflection point of the oxygenation index might have a physiological function only under severe metabolic conditions, because there was an approximate twofold difference in oxygenation level among the portions at the inflection point (see Fig. 2). Although a reverse slope change in oxygenation index (i.e., from steeper to more shallow) was observed only in subject 4, the inflection point of the oxygenation index was correlated with both cardiovascular responses (Fig. 2). This finding might suggest that the inflection point of oxygenation index indicates an onset of critical oxygenation level at which aerobic metabolism becomes inhibited. Therefore the critical oxygenation level might be important in interpreting the physiological processes reflected in the inflection point of the oxygenation index. However, because of methodological limitation of the NIRS device, we could measure only relative and not absolute (i.e., quantitative) changes in muscle oxygenation. Thus we cannot completely explain the precise physiological processes reflected in the inflection point of muscle oxygenation in exercising muscle. Further investigation is needed to clarify the reasons for the regional differences in the inflection points between muscle portions while considering the relationship between the critical level of muscle oxygenation and the pressor response.

In conclusion, our data show that the inflection point of muscle oxygenation occurred at a different workload in proximal and distal portions of the vastus lateralis muscle. The inflection point of muscle oxygenation was highly correlated with the inflection point of cardiovascular responses during incremental exercise in the distal but not in the proximal portion. Our data also suggest that the distal portion of the vastus lateralis muscle makes a greater contribution to the pressor response than does the proximal portion.

REFERENCES


