Central command contributes to increased blood flow in the noncontracting muscle at the start of one-legged dynamic exercise in humans

Kei Ishii,1 Nan Liang,1 Anna Oue,2 Ai Hirasawa,2 Kohei Sato,2 Tomoko Sadamoto,2 and Kanji Matsukawa1

1Department of Physiology, Graduate School of Health Sciences, Hiroshima University, Hiroshima, Japan; and 2Research Institute of Physical Fitness, Japan Women’s College of Physical Education, Tokyo, Japan

Submitted 17 January 2012; accepted in final form 10 April 2012

Ishii K, Liang N, Oue A, Hirasawa A, Sato K, Sadamoto T, Matsukawa K. Central command contributes to increased blood flow in the noncontracting muscle at the start of one-legged dynamic exercise in humans. J Appl Physiol 112: 1961–1974, 2012. First published April 12, 2012; doi:10.1152/japplphysiol.00075.2012.—Whether neurogenic vasodilatation contributes to exercise hyperemia is still controversial. Blood flow to noncontracting muscle, however, is chiefly regulated by a neural mechanism. Although vasodilatation in the nonexercising limb was shown at the onset of exercise, it was unclear whether central command or muscle mechanoreflex is responsible for the vasodilatation. To clarify this, using voluntary one-legged cycling with the right leg in humans, we measured the relative changes in concentrations of oxygenated-hemoglobin (Oxy-Hb) of the noncontracting vastus lateralis (VL) muscle with near-infrared spectroscopy as an index of tissue blood flow and femoral blood flow to the nonexercising leg. Oxy-Hb in the noncontracting VL and femoral blood flow increased ($P < 0.05$) at the start period of voluntary one-legged cycling without accompanying a rise in arterial blood pressure. In contrast, no increases in Oxy-Hb and femoral blood flow were detected at the start period of passive one-legged cycling, suggesting that muscle mechanoreflex cannot explain the initial vasodilatation of the noncontracting muscle during voluntary one-legged cycling. Motor imagery of the voluntary one-legged cycling increased Oxy-Hb of not only the right but also the left VL. Furthermore, an increase in Oxy-Hb of the contracting VL, which was observed at the start period of voluntary one-legged cycling, had the same time course and magnitude as the increase in Oxy-Hb of the noncontracting muscle. Thus it is concluded that the centrally induced vasodilator signal is equally transmitted to the bilateral VL muscles, not only during imagery of exercise but also at the start period of voluntary exercise in humans.

vasodilatation; muscle mechanoreflex; near-infrared spectroscopy; ultrasound Doppler flowmetry

Whether neurogenic vasodilatation contributes to exercise hyperemia is still controversial. Our laboratory recently reported that a sympathetic cholinergic mechanism is capable of evoking exercise hyperemia at the onset of voluntary static exercise in conscious cats (31). Skeletal muscle vasodilatation via the sympathetic cholinergic system has been observed during the defense reaction or classical conditioning task (2, 3, 19). Other literature, however, indicates that the sympathetic cholinergic mechanism is not essential for the rapid vasodilatation in active skeletal muscle at the beginning of exercise, because surgical sympathectomy and ganglionic or muscarinic blockade had little influence on increased blood flow to the exercising limb after a brief forearm contraction (9, 14) and at the onset of intermittent handgrip exercise in humans (48) and during treadmill exercise in dogs (10). The discrepancy about a role of sympathetic vasodilatation at the onset of exercise may be attributed to the fact that neurogenic vasodilatation occurs during a more voluntary type of exercise with greater volitional effort and is masked by other vasodilator mechanisms, such as metabolic or flow-mediated vasodilatation.

In contrast to contracting muscle, a neural mechanism via the sympathetic nervous system appears to regulate blood flow of noncontracting muscle, because muscle contraction is absent, and no metabolites are released in the muscle. In general, it has been thought that an increase in sympathetic adrenergic vasoconstrictor outflow restricts blood flow in noncontracting muscles during the later phase of exercise (more than $\sim 2$ min after the exercise onset) (7, 27, 33, 42). However, other studies demonstrated transient increases in blood flow and vascular conductance of the nonexercising limb at the initial phase of static or dynamic exercise (until $\sim 1–2$ min from the exercise onset) (5, 18, 23, 50). The initial vasodilator response depended on a relative force of contraction rather than on a mass of the muscles involved (17, 53). These results suggest that blood flow in noncontracting skeletal muscle may increase at the start of exercise, and the vasodilatation is evoked by a neurogenic mechanism in association with volitional effort. First of all, it is conceivable that central command is responsible for vasodilatation in the noncontracting muscle at the start of voluntary exercise, because mental stress (6, 16, 41), immobile alerting and fighting behavior (2, 3, 19), and electrical or chemical stimulation of neurons in some regions of the hypothalamus and midbrain in animal preparation elicit an increase in skeletal muscle blood flow (1, 4, 12, 25, 26, 30, 34–36, 39). Sanders et al. (44) observed that the voluntary isometric handgrip exercise with one arm induced a decrease in vascular resistance of the other arm, which was blocked by atropine but not by propranolol. This finding suggests that centrally induced activation of the sympathetic cholinergic system plays a role in increasing blood flow to the noncontracting muscle in humans. Next, exercise pressor reflex from the contracting muscle may cause vasodilatation in the contralateral limb. In particular, muscle mechanoreflex will be added as a possible candidate responsible for the initial vasodilatation, because the passive knee-extension exercise slightly increased femoral blood flow of the nonexercising limb (52). On the other hand, posthandgrip ischemia caused vasoconstriction of the calf vascular bed, instead of vasodilatation (46), suggesting that muscle metaboreflex is unlikely to play a role in the neurogenic vasodilatation in the noncontracting muscle.
To clarify which of the two mechanisms (central command and muscle mechanoreflex) plays a more important role in the initial neurogenic vasodilatation of the noncontracting muscle, we compared the relative changes in concentration of oxygenated-hemoglobin (Oxy-Hb) in the noncontracting vastus lateralis (VL) muscle with near-infrared spectroscopy (NIRS) as an estimate of muscle tissue blood flow and femoral blood flow to the nonexercising leg with Doppler ultrasound between voluntary and passive one-legged ergometer exercise. As a result, Oxy-Hb in the noncontracting VL muscle and femoral blood flow increased at the start of the voluntary cycling but not the passive cycling, suggesting that central command is the sole candidate for the initial neurogenic vasodilatation in noncontracting muscle. To further test the hypothesis, we examined the responses of Oxy-Hb in the noncontracting muscle during imagery of the voluntary one-legged exercise. The descending vasomotor outflow by central command would be transmitted, not only to the nonexercising limb but also to the exercising limb during one-legged exercise, because a recent study demonstrated that many medullary vasomotor neurons were dually infected by recombinants of pseudorabies virus injected into the bilateral hindlimbs of rats (32). To test the second hypothesis, we compared the responses of Oxy-Hb in the bilateral VL muscles not only during the voluntary one-legged exercise but also during motor imagery of the exercise. A part of this study has been published in preliminary form (28).

METHODS

Subjects

Twelve healthy volunteers (eight males and four females; age, 24 ± 1 yrs; height, 170 ± 2 cm; body wt, 58 ± 2 kg) participated in the present study. None of the subjects suffered from any known cardiovascular and neuromuscular diseases or took any medications. The experimental procedures and protocols were performed in accordance with the Declaration of Helsinki and approved by the Institutional Ethical Committee. The subjects gave their informed, written consent prior to the experiments. All experiments were performed in a thermoneutral and soundproof environment, and all were conducted at Hiroshima University.

One-Legged Cycling Exercise

The subjects lay down in the supine position on a comfortable reclining seat of a specially designed cycle ergometer (Strength Ergo 240 BK-ERG-003, Mitsubishi Electric Engineering, Tokyo, Japan) and performed voluntary one-legged exercise with the right leg. The ergometer enabled passive one-legged cycling, driven with a motor. The right foot was put on a specially designed shoe, affixed at the pedal. The positions of the crank, pedal, and seat were adjusted so as to allow the subjects to remain in a comfortable and certain posture. The subjects were instructed to perform one-legged isotonic cycling with the right leg alone and to keep the left leg relaxed throughout the experiments. Torque against the wheel shaft and pedal displacement of the ergometer were measured continuously. We confirmed that electrical activity of the left VL muscle was absent during the one-legged exercise (Fig. 1).

To determine the maximal intensity of voluntary one-legged exercise, the subjects conducted an incremental one-legged exercise test on a separate day prior to the main experiments. One-legged cycling with the right leg was performed for 1 min at 50 rpm, starting at an intensity of 8 Nm for males and 3 Nm for females and increasing the workload by 4 Nm for males and by 3 Nm for females. The interval between bouts was set at 3–5 min. The incremental bout was continued until the subjects could no longer perform the cycling or maintain the required revolution speed. The maximal exercise that the subjects completed was defined as the maximal voluntary effort.

Measurements of Muscle and Skin Tissue Blood Flow

The relative concentrations of Oxy-Hb and deoxygenated-hemoglobin (Deoxy-Hb) in the left VL muscle were measured with NIRS, while skin blood flow over the muscle was recorded with Laser Doppler Flowmetry. The basic principle of NIRS is that near-infrared light, emitted from three laser photodiodes with different wavelengths, penetrates skeletal muscle tissue and that some of the light is absorbed by Hb, myoglobin (Mb), and cytochromes, and others, scattered by the tissue, are picked up with photodetectors (8, 21, 37). Seiyama et al. (47) reported that the total contribution of Mb and cytochromes to the signals of NIRS is <10% in isolated rat skeletal muscle, indicating that the Hb in blood vessels of muscle tissue chiefly affects the signals of NIRS. The muscle oxygenation signals of NIRS are dependent on a balance of oxygen supply and use in the tissue. As long as oxygen use is constant and at the minimum (e.g., noncontracting muscle), the signal of Deoxy-Hb will be constant, and the signal of Oxy-Hb may reflect muscle tissue blood flow. We monitored the Oxy-Hb as an estimate of tissue blood flow in noncontracting skeletal muscle. A pair of photoemission and photodetection probes was placed two-thirds from the greater trochanter to the top of the patella and attached 4 cm apart on the skin over the left VL muscle so that near-infrared light intersected the muscle bundles. The reflected near-infrared light (wavelengths: 775 nm, 810 nm, and 850 nm), through muscle tissue, was sampled at a rate of 6 Hz and converted to optical densities with a near-infrared spectrometer (NIR-200, Hamamatsu Photonics, Hamamatsu, Japan).

To clarify the contribution of skin blood flow to the signals of NIRS, skin blood flow was monitored with a Laser Doppler instrument (ALF21, Advance, Tokyo, Japan). A Doppler flow probe was placed on the left VL muscle surface adjacent to the NIRS probes. The Doppler flow signal was time averaged with a time constant of 0.1 s.

Measurements of Femoral Blood Flow

Blood flow of the left femoral artery was measured with a high-resolution ultrasound Doppler instrument (Vivid e, GE Healthcare, Tokyo, Japan). A 10-MHz linear probe was placed below the inguinal ligament and ~2–3 cm above the bifurcation into the profundus and superficial branches (49). Blood flow velocity measurements were performed with the insonation angle <60° and were corrected for the insonation angle. Care was taken to ensure that the probe position and the insonation angle were appropriate and stable and that the sample volume was positioned in the center of the vessel and adjusted to cover the width of the vessel diameter. Ultrasound B-mode images were used to measure systolic and diastolic internal diameter of the femoral artery. A pulsed Doppler mode was used to measure femoral blood flow velocity. The mean internal diameter was calculated based on the following equation: mean internal diameter = [(systolic internal diameter × 1/3)] + [(diastolic internal diameter × 2/3)], as reported by Sato et al. (45). The time-averaged mean blood flow velocity over 5 s was defined as mean blood flow velocity. Femoral blood volume flow was calculated by multiplying the cross-sectional area [π × (mean internal diameter/2)²] by the mean blood flow velocity.

Cardiovascular and Electromyogram Recordings

A pair of electrodes (Magnerode, TE-18, Fukuda Denshi, Tokyo, Japan) and a ground electrode were attached on the chest for measuring ECG. The ECG signal and respiratory movement were monitored with a telemetry system (DynaScope DS-3140, Fukuda Denshi). Arterial blood pressure (AP) was measured noninvasively and con-
Fig. 1. A: representative recordings of motor performance and the cardiovascular and electromyogram (EMG) responses in transition from rest to voluntary 1-legged cycling with an intensity of 33% of the maximal effort in a subject. The exercise onset was defined as the onset of pedal displacement (as indicated by an arrow). Concentration of oxygenated-hemoglobin (Oxy-Hb) in the noncontracting vastus lateralis (VL) muscle and heart rate (HR) increased at the start of voluntary 1-legged cycling with no distinct changes in deoxygenated-Hb (Deoxy-Hb), skin blood flow, and arterial blood pressure (AP). B: the average integrated EMG activity of the contracting (●) and noncontracting (○) VL muscle during voluntary 1-legged cycling (n = 7 subjects). Vertical, dashed lines indicate the start and end of 1-legged cycling. Note that no EMG activity was observed in the noncontracting VL muscle during voluntary 1-legged cycling.
signals were recorded in the left (exercise (circle-imagery) for 1 min after a cue was given. The NIRS exercise. As control, the subjects imagined a circle with no relation to imagery was performed soon after execution of voluntary one-legged cycling exercise (cy-
portion of the muscle belly. Prior to EMG electrode application, skin was cleaned up with alcohol and preparatory gel (skinPure, Nihon Kohden, Tokyo, Japan). The EMG signals were amplified (×10,000) and filtered with a bandpass filter between 20 and 2,000 Hz (Bagnoli-2 EMG system, Delsys, Boston, MA).

Experimental Protocols

Motor performance (developed torque and pedal displacement of the ergometer), the cardiovascular responses, and the EMG activities of the bilateral VL muscles were recorded simultaneously in all subjects throughout each individual protocol of voluntary and passive one-legged cycling and cycling- and circle-imagery. The EMG activity of the noncontracting muscle(s) was absent in all cases (as exemplified in Fig. 1B).

Protocol 1: NIRS measurements during voluntary and passive one-legged exercise. Seven subjects performed voluntary one-legged cycling with the right leg for 1 min at 50 rpm, which was started freely without any cue. The exercise intensity was set 8 Nm for six males and 5 Nm for one female, which corresponded to 29–37% (average, 34 ± 1%) of the maximal voluntary effort. To isolate an influence of muscle mechanoreflex on muscle blood flow, passive one-legged cycling with the right leg was driven by a motor of the ergometer for 1 min at 50 rpm without any cue and volitional effort in the same subjects. The NIRS signals of the noncontracting left VL muscle were measured during voluntary and passive one-legged cycling in all subjects. Skin blood flow on the VL muscle was measured in five of the subjects. The NIRS signals of the contracting and noncontracting VL muscles were recorded during voluntary and passive one-legged cycling in seven subjects (five of the original seven subjects and newly recruited two subjects).

Protocol 2: Doppler ultrasound flowmetry during voluntary and passive one-legged exercise. Femoral blood flow to the nonexercising left leg was measured during voluntary and passive one-legged cycling in six subjects (three subjects participated in Protocol 1 and newly recruited three subjects). To minimize movement artifact in measurement of femoral blood flow, the exercise intensity of cycling was reduced to 5 Nm for three males and 3 Nm for three females, which corresponded to 16–21% (average, 18 ± 1%) of the maximal voluntary effort.

Protocol 3: motor imagery of voluntary one-legged exercise. To examine the pure influence of central command on muscle blood flow without any feedback from contracting muscle, seven subjects (five subjects participated in Protocol 1, and two subjects participated in Protocol 2) were asked to imagine one-legged cycling exercise (cycling-imagery) for 1 min, as soon as a cue was given. Cycling-imagery was performed soon after execution of voluntary one-legged exercise. As control, the subjects imagined a circle with no relation to exercise (circle-imagery) for 1 min after a cue was given. The NIRS signals were recorded in the left (n = 7) and right VL muscle (n = 5) during cycling- and circle-imagery protocols. Femoral blood flow to the nonexercising left leg was measured during each imagery protocol in all seven subjects.

Data Analysis

The data of AP, ECG, EMG, NIRS signals, and skin blood flow were simultaneously stored to computers at a sampling frequency of 1,000 Hz (MP150, Biopack Systems, Santa Barbara, CA, and PowerLab system, ADInstruments, Castle Hill, Australia) for offline analysis. To calculate the integrated EMG, the raw EMG signal was processed with full-wave rectification, and a moving average of the rectified EMG was conducted over neighboring 1,000 points. The integrated EMG was averaged sequentially every 1 s. The changes in Oxy-Hb, HR, SV, CO, MAP, and TPR from the baseline were averaged sequentially every 1 s. The average values of mean internal diameter of the femoral artery and femoral blood flow velocity and volume flow over a period of 5 s were calculated sequentially. Femoral vascular conductance was calculated by dividing femoral blood flow by MAP at each period. Since the pathlength of the near-infrared light within the tissue was unknown in vivo, it was difficult to determine the absolute concentration of Oxy-Hb. Instead, we expressed the relative change in Oxy-Hb as a percentage against the baseline control. When a pneumatic cuff, wrapped around the upper thigh, was inflated with a pressure of 200–250 mmHg, the minimum value of Oxy-Hb was defined as zero. Since the NIRS signals in the contracting VL muscle involved movement artifact during voluntary one-legged cycling, the data were recalculated by conducting a moving average over neighboring 1,000 points. In the imagery protocols, the responses of all variables were obtained as an average over a time period from 30 to 45 s after the imagery onset. To estimate the vividness of imagery, we used a visual analog scale (VAS) score from 0 (not vivid at all) to 10 (the most vivid), as described by Williamson et al. (51).

Statistical Analysis

The time course data of the cardiovascular and femoral blood flow responses and the NIRS signals during one-legged cycling were statistically analyzed by a one-way ANOVA with repeated measures. If a significant F value in the main effect of time were present, a Bonferroni post hoc test was performed to detect a significant difference in mean values at a given time from the baseline control. The effects of exercise type (voluntary vs. passive) or imagery type (cycling vs. circle-imagery) on the cardiovascular and femoral blood flow responses and the NIRS signals were analyzed by a two-way ANOVA with repeated measures. The mean values obtained during the later period of cycling exercise or imagery were compared with the baseline values by a paired t-test. A level of statistical significance was defined at P < 0.05 in all cases. All parameters are expressed as means ± SE.

RESULTS

The Cardiovascular Responses During Voluntary One-Legged Cycling

The representative recordings of motor performance and the cardiovascular and EMG responses in transition from rest to voluntary one-legged cycling are shown in Fig. 1A. As soon as the subject started cycling, the pedal was displaced, and torque was generated. Periodic muscular activity in the contracting right VL muscle was observed, whereas EMG of the noncontracting left VL muscle was absent throughout the voluntary cycling (Fig. 1A and B).

Fig. 1A shows that HR increased abruptly at the start period of the voluntary cycling, whereas AP was unchanged at that period. The absolute values of the hemodynamics before and during voluntary one-legged cycling are summarized in Table 1. The time courses of the average cardiovascular responses
The hemodynamic responses during voluntary and passive 1-legged cycling

<table>
<thead>
<tr>
<th></th>
<th>Voluntary 1-legged cycling</th>
<th>Passive 1-legged cycling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>During</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>63 ± 2.9</td>
<td>100 ± 2.4*†</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>96 ± 6.2</td>
<td>103 ± 7.5*</td>
</tr>
<tr>
<td>CO (/min)</td>
<td>60 ± 0.4</td>
<td>103 ± 0.7*†</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>97 ± 5.1</td>
<td>108 ± 4.8*†</td>
</tr>
<tr>
<td>TPR (mmHg · min⁻¹)</td>
<td>16.8 ± 1.7</td>
<td>11.0 ± 1.1*†</td>
</tr>
</tbody>
</table>

Baseline hemodynamic values were not significantly different (P > 0.05) between voluntary and passive 1-legged cycling in 7 subjects. HR, heart rate; SV, stroke volume; CO, cardiac output; MAP, mean arterial blood pressure; TPR, total peripheral resistance. The average responses in HR, CO, MAP, and TPR during passive cycling were smaller (P < 0.05) as compared with those during voluntary cycling. *Significant difference (P < 0.05) from the baseline before the exercise; †significant difference (P < 0.05) between voluntary and passive cycling. Values are means ± SE.

The responses in femoral blood flow during voluntary and passive one-legged cycling

When the exercise intensity of voluntary one-legged cycling was reduced to 18 ± 1% of the maximal effort, the responses of MAP and TPR (Fig. 4) tended to be smaller compared with those during voluntary one-legged cycling with the higher intensity (as shown in Fig. 2). Femoral blood flow velocity increased by 60% (P < 0.05, from 14.9 ± 1.7 to 23.8 ± 2.4 cm/s) during voluntary cycling at 15–45 s from the exercise onset, whereas mean internal diameter of the femoral artery did not change from the baseline of 0.81 ± 0.06 cm. Thus in proportion to femoral blood flow velocity, femoral blood flow increased by 50% (P < 0.05, from 495 ± 115 to 744 ± 138 ml/min) at 15–45 s from the exercise onset. A significant increase of 48% in femoral vascular conductance (from 4.8 ± 1.1 to 7.1 ± 1.2 ml·min⁻¹·mmHg⁻¹) was also detected. The vasodilator response was observed consistently in each individual during voluntary one-legged cycling (Fig. 5A). Furthermore, there was a good temporal coincidence between the increases in femoral vascular conductance (Figs. 4 and 5) and Oxy-Hb (Fig. 3), although the exercise intensity was lower in Doppler ultrasound flowmetry.

In contrast to voluntary one-legged cycling, femoral internal diameter, blood flow velocity, blood flow, and vascular conductance were unchanged (P > 0.05) from the baseline during passive one-legged cycling (Fig. 4). Accordingly, there were significant differences (P < 0.05 by a two-way ANOVA) in the responses of femoral blood flow and vascular conductance, as well as TPR, between passive and voluntary one-legged exercise (Fig. 4). However, an increase in femoral vascular conductance was observed in three of the subjects at the later period (25–60 s) of passive cycling (Fig. 5B). The vasodilator response corresponded with the delayed increase in Oxy-Hb during passive cycling (Fig. 3). In the remaining subjects, femoral vascular conductance did not change throughout the passive cycling.

The influence of motor imagery on the Oxy-Hb and femoral blood flow responses

To identify any influence of central command on muscle blood flow, we measured hemodynamics and the responses in femoral blood flow and the NIRS signals during motor imagery of voluntary one-legged cycling, as shown in Fig. 6. Cycling-imagery induced an increase in Oxy-Hb of the left VL muscle without changing Deoxy-Hb (Fig. 6A). HR increased slightly,
but AP did not change during cycling-imagery. Femoral blood flow velocity increased without a change in mean internal diameter of the femoral artery. Thus central command involved in cycling-imagery was able to cause tachycardia and hyperemia in skeletal muscle. In contrast, when the subject imagined a circle with no relation to exercise, no distinct changes in any variables were observed (Fig. 6B).

The absolute values of the hemodynamics and femoral blood flow during cycling- and circle-imagery are summarized in Table 2. The relative changes in Oxy-Hb of the right and left VL muscles and the femoral blood flow response are compared between the two imagery conditions in Fig. 7. During cycling-imagery, HR significantly ($P < 0.05$) but slightly increased, whereas SV, CO, MAP, and TPR remained unchanged (Table 2). Motor imagery of voluntary one-legged cycling also increased ($P < 0.05$)—not only Oxy-Hb of the contralateral left VL but also Oxy-Hb of the ipsilateral right VL (Fig. 7A). It was of interest that the increase of Oxy-Hb during cycling-imagery was equal between the left and right VL ($3.1 \pm 0.6\%$ vs. $3.1 \pm 0.3\%$, respectively). Corresponding to the increase in Oxy-Hb of the left VL, femoral blood flow velocity, volume flow, and vascular conductance increased ($P < 0.05$) during cycling-imagery with no changes in mean internal diameter of the femoral artery (Fig. 7B and Table 2). In contrast, Oxy-Hb of the bilateral VL muscles failed to change during circle-imagery (Fig. 7A), although the VAS score rating the vividness of imagery was not different ($P > 0.05$) between the two imagery conditions (Fig. 7C). Furthermore, circle-imagery elicited no

![Fig. 2. The time courses of the cardiovascular responses during voluntary (○) and passive (●) 1-legged cycling in 7 subjects. Each value was calculated sequentially every 1 s. Vertical, dashed lines indicate the start and end of 1-legged cycling, whereas a vertical, dotted line is placed at 15 s from the exercise onset. HR and cardiac output (CO) increased, and total peripheral resistance (TPR) decreased at the start of voluntary 1-legged cycling, and the changes were sustained until the cessation of the exercise. In contrast, HR and CO increased slightly, and TPR decreased slightly, only during the later period (12–60 s) of passive 1-legged cycling. SV, stroke volume; MAP, mean AP. *Significant difference from the baseline ($P < 0.05$) in the data during voluntary 1-legged cycling; †significant difference from the baseline ($P < 0.05$) in the data during passive 1-legged cycling.](http://jap.physiology.org/)

![Fig. 3. The time courses of the relative changes in Oxy-Hb and Deoxy-Hb of the noncontracting VL muscle during voluntary (○) and passive (●) 1-legged cycling in 7 subjects. The relative percent change in Oxy-Hb was determined by identifying the 0 level with muscle ischemia. Each value was calculated sequentially every 1 s. Vertical, dashed lines indicate the start and end of 1-legged cycling, whereas a vertical, dotted line is placed at 15 s from the exercise onset. Oxy-Hb began to increase ($P < 0.05$) at 7–8 s from the onset of voluntary 1-legged cycling, whereas Deoxy-Hb unchanged ($P > 0.05$) throughout the exercise. In contrast, Oxy-Hb and Deoxy-Hb failed to change ($P > 0.05$) at the start of passive 1-legged cycling. *Significant difference from the baseline ($P < 0.05$) in the data during voluntary 1-legged cycling; †significant difference from the baseline ($P < 0.05$) in the data during passive 1-legged cycling.](http://jap.physiology.org/)
significant increases ($P > 0.05$) in femoral blood flow velocity, volume flow, vascular conductance, and mean internal diameter. Also, none of the hemodynamic variables changed ($P > 0.05$) from the baseline during circle-imagery (Table 2).

**The Responses in Oxy-Hb of the Contracting vs. Noncontracting Muscle During Voluntary One-Legged Cycling**

Figure 8 shows a typical example of the raw NIRS signals of the right (contracting) and left (noncontracting) VL muscles during voluntary one-legged cycling without conducting a moving average. Although the NIRS signals of the right VL muscle contained artifacts due to limb movement, Oxy-Hb in the bilateral VL muscles seemed to increase at the start of voluntary cycling without changing Deoxy-Hb. In particular, the initial increase in Oxy-Hb of the right (contracting) VL muscle was followed by a subsequent decrease in Oxy-Hb and an increase in Deoxy-Hb.

The time courses of the changes in moving-averaged Oxy-Hb and Deoxy-Hb of both contracting and noncontracting VL muscles during voluntary one-legged cycling are summa-
Central Command and Muscle Blood Flow • Ishii K et al.

**DISCUSSION**

This is the first study using NIRS to suggest whether central command contributes to increased blood flow in noncontracting muscle at the start of voluntary one-legged exercise in humans. The major new findings of the present study are that 1) Oxy-Hb in the noncontracting VL muscle increased at the start period of voluntary one-legged cycling with no changes in Deoxy-Hb and skin blood flow, and the initial increase in Oxy-Hb was probably due to increased blood flow in noncontracting muscle; 2) Oxy-Hb in the noncontracting VL was unchanged at the start period of passive cycling and thereafter increased; 3) the increases in femoral blood flow and vascular conductance in the nonexercising limb were observed at the start period of voluntary one-legged cycling but not passive cycling; 4) Oxy-Hb in the bilateral VL muscles did not alter during circle-imagery but increased during motor imagery of the voluntary cycling without changing Deoxy-Hb; and 5) Oxy-Hb of the contracting VL muscle increased at the start period of voluntary one-legged cycling with the same time course and magnitude as the Oxy-Hb response of the noncontracting VL. Taken together, it is likely that central command chiefly contributes to the initial vasodilation in noncontracting muscle during voluntary exercise and that the neurogenic vasodilation may occur in the contracting muscle as well.

**Evaluation of Muscle Blood Flow with NIRS and Ultrasound Doppler Flowmetry**

The present evaluation of muscle tissue blood flow with NIRS involves fundamental assumptions and limitations. As mentioned before, the relative changes in the NIRS signals chiefly reflect the changes in near-infrared light, partly absorbed by Oxy- and Deoxy-Hb in muscle tissues, because the total contribution of Mb and cytochromes to the signals of NIRS is <10% in isolated rat skeletal muscle (47). The muscle oxygenation signals of NIRS are dependent on a balance of oxygen supply and use in the tissue. As long as oxygen use is at the minimum, the signal of Deoxy-Hb will be constant, and the signal of Oxy-Hb may reflect muscle tissue blood flow. Indeed, the Oxy-Hb increased during one-legged cycling and during imagery of the voluntary cycling without accompanying any significant changes in the Deoxy-Hb. Although the NIRS signals are influenced by skin blood flow as well as muscle blood flow, no changes in skin blood flow were observed during one-legged cycling (Fig. 1A). Accordingly, we assumed that the relative percent change in Oxy-Hb can be taken as an index of muscle tissue blood flow. The relative changes in Oxy-Hb of the forearm and triceps surae muscles are known to correlate linearly with the changes in limb blood flow during lower-body negative pressure (20) and during isometric exercise in humans (38). Since the NIRS signals is strongly influenced by a content of Hb, a change in tissue blood volume due to periodic limb movement caused artifacts in the NIRS signals of the contracting VL muscle. To cancel the artifacts, the mean values of the NIRS signals were calculated by conducting a moving average over neighboring 1,000 points. To what extent limb movement affected the mean NIRS data was evaluated by examining the effect of passive one-legged cycling on the NIRS signals. Since mean Oxy-Hb was unchanged at the start of passive cycling and subsequently, increased with the same time course and magnitude as the Oxy-Hb in the noncontracting muscle, the initial increase in mean Oxy-Hb of the contracting VL muscle is unlikely to result from an artifact due to limb movement.

**Fig. 5.** The time courses of the changes in femoral vascular conductance responses during voluntary (A) and passive (B) 1-legged cycling in 6 subjects. Each solid line indicates the time course data of femoral vascular conductance taken from each subject. The vasodilator response was observed consistently in every individual during voluntary 1-legged cycling but not passive 1-legged cycling.
Also, there are some potential limitations with Doppler flowmetry. First, since the exercise intensity of voluntary one-legged cycling was reduced to minimize a movement artifact on the Doppler signal, it cannot be denied that the blood flow response of the femoral artery might be underestimated compared with the NIRS data. Second, the NIRS signals were not measured simultaneously with femoral blood flow during voluntary cycling, because an attempt to keep the ultrasound probe at an appropriate position usually caused artifacts for the NIRS signals. Third, it may be difficult to compare the blood flow responses obtained from Doppler ultrasound and NIRS, because femoral blood flow contains not only blood flow to the entire musculature downstream from the femoral artery but also blood flow to cutaneous tissues, whereas the NIRS data are focused on the vasculature involved in a localized region of the VL muscle and skin. Nevertheless, it is conceivable that a change in femoral blood flow follows a change in blood flow to the quadriceps muscle, because skin blood flow did not change during one-legged exercise, and the quadriceps muscle has the greatest mass in the limb.

Table 2. The responses in hemodynamics and femoral blood flow during cycling- and circle-imagery

<table>
<thead>
<tr>
<th></th>
<th>Cycling-imagery</th>
<th>Circle-imagery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>During</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>67 ± 3.0</td>
<td>70 ± 2.7*</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>85 ± 7.4</td>
<td>84 ± 7.5</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>5.6 ± 0.3</td>
<td>5.8 ± 0.3</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>95 ± 2.9</td>
<td>99 ± 3.3</td>
</tr>
<tr>
<td>TPR (mmHg · min · 1⁻¹)</td>
<td>17.4 ± 1.0</td>
<td>17.6 ± 1.2</td>
</tr>
<tr>
<td>Mean internal diameter of femoral artery (cm)</td>
<td>0.84 ± 0.02</td>
<td>0.85 ± 0.02</td>
</tr>
<tr>
<td>Femoral blood flow velocity (cm/s)</td>
<td>14.8 ± 2.4</td>
<td>17.6 ± 3.2*</td>
</tr>
<tr>
<td>Femoral blood flow (ml/min)</td>
<td>498 ± 90</td>
<td>598 ± 115*</td>
</tr>
<tr>
<td>Femoral vascular conductance (ml · min⁻¹ · mmHg⁻¹)</td>
<td>5.1 ± 1.0</td>
<td>6.1 ± 1.3*</td>
</tr>
</tbody>
</table>

*Significant difference (P < 0.05) from the baseline before the imagery. Values are means ± SE.
Vasodilatation in Noncontracting Muscle at the Onset of Voluntary Exercise

In agreement with previous studies (5, 18, 23, 50), CO and femoral blood flow in the nonexercising leg were increased at the start of voluntary one-legged cycling. The increase in CO might contribute to increased blood flow to the nonexercising limb without a change in arterial contractile state. Femoral blood flow, however, increased at the start of one-legged exercise without any rise in MAP, and calculated vascular conductance actually increased at 15–45 s during voluntary one-legged exercise (Fig. 4), indicating vasodilatation in the contralateral leg. Furthermore, cycling-imagery increased femoral blood flow without changing CO, suggesting that the hyperemic response is mediated with vasodilatation, presumably caused by a descending signal from higher brain centers. Taken together, increased blood flow to the femoral artery is not simply due to an increase in systemic perfusion pressure and/or CO but is associated with vasodilatation in the nonexercising limb. In addition, the internal diameter of the femoral artery was unchanged during either imagery. *Significant difference from the baseline (P < 0.05); †significant difference (P < 0.05) between cycling- and circle-imagery protocol. N.S., not significant.

Fig. 7. The effects of cycling- and circle-imagery on the responses in Oxy-Hb of the bilateral VL muscles and femoral blood flow and the extent of vividness of imagery. A: the average percent changes in Oxy-Hb of the right (n = 5 subjects) and left (n = 7 subjects) VL muscle. B: the average changes in mean internal diameter of the femoral artery, femoral blood flow velocity, femoral blood flow, and femoral vascular conductance (n = 7 subjects). C: the visual analog scale (VAS) score rating the vividness of imagery (n = 7 subjects). Cycling-imagery, but not circle-imagery, increased (P < 0.05) Oxy-Hb in the bilateral VL muscles to the same extent, although the VAS score rating the vividness of imagery was not different (P > 0.05) between the 2 imagery protocols. Femoral blood flow velocity, blood flow, and vascular conductance increased (P < 0.05) during cycling-imagery, whereas they failed to change during circle-imagery. The femoral internal diameter was unchanged during either imagery. *Significant difference from the baseline (P < 0.05); †significant difference (P < 0.05) between cycling- and circle-imagery protocol. N.S., not significant.

![Graphs showing changes in Oxy-Hb and femoral blood flow during cycling- and circle-imagery.](http://jap.physiology.org/)

---

J Appl Physiol • doi:10.1152/japplphysiol.00075.2012 • www.jappl.org
vasodilatation in the noncontracting skeletal muscle contributes to the increase in upstream femoral blood flow. Since EMG of the noncontracting VL was absent during voluntary one-legged cycling (Fig. 1), the increase in flow cannot be attributed to metabolic vasodilatation due to inadvertent muscle contraction. Thus the initial hyperemia in the noncontracting VL muscle may be explained by flow-mediated vasodilatation via increased perfusion and/or neurogenic vasodilatation. An increase in blood flow produces shear stress-induced release of nitric oxide (NO) and prostacyclin from the endothelium, which in turn, causes flow-mediated vasodilatation (13). The contribution of flow-mediated vasodilatation to the initial hyperemia in the noncontracting VL was unknown in this study, because we did not examine the effects of inhibitors of endothelial NO and/or prostacyclin production on the initial hyperemia in the noncontracting muscle. However, it seems that the flow-mediated vasodilatation hardly explains the rapid hyperemia immediately after the onset of voluntary one-legged cycling, because Koller and Kaley (29) demonstrated that an increase in red blood cell velocity per se induced an increase in the diameter of arterioles with a substantial time lag in the cremaster muscle of the anesthetized rat. Furthermore, when comparing the NIRS data and femoral blood flow with the same time frame, the increase in femoral blood flow did not precede the rise in Oxy-Hb, indicating that the increase in upstream femoral blood flow was not responsible for the initial vasodilatation in the noncontracting muscle. Taken together, neurogenic vasodilatation seems the sole candidate responsible for the initial hyperemia in the noncontracting muscle. A possibility that a higher intensity and a longer duration of one-legged exercise may modify the response in blood flow to

Fig. 8. A typical example of the raw near-infrared spectroscopy (NIRS) signals of the right (contracting) and left (noncontracting) VL muscle during voluntary 1-legged cycling in a subject. Vertical, dashed lines indicate the start and end of 1-legged cycling, whereas a vertical, dotted line is placed at 15 s from the exercise onset. The NIRS signals of the right VL muscle contained artifacts due to limb movement. *Peak increases in Oxy-Hb of the bilateral VL muscles at the start period (0–15 s) of voluntary 1-legged cycling.

Fig. 9. The time courses of the relative changes in moving-averaged Oxy-Hb and Deoxy-Hb of the contracting (●) and noncontracting (○) VL muscle during voluntary 1-legged cycling in 7 subjects. Each value was calculated sequentially every 1 s. Vertical, dashed lines indicate the start and end of 1-legged cycling, whereas a vertical, dotted line is placed at 15 s from the exercise onset. At the start of voluntary 1-legged exercise, mean Oxy-Hb in the contracting and noncontracting VL muscles increased with almost the same time course and magnitude; there was no significant difference in the Oxy-Hb response between the 2 muscles (P > 0.05, a 2-way ANOVA). Subsequently, mean Oxy-Hb decreased, and mean Deoxy-Hb increased in the contracting VL, as long as exercise was continued. **Significant difference from the baseline (P < 0.05) in the noncontracting VL data at the start period of 1-legged exercise; †significant difference from the baseline (P < 0.05) in the contracting VL data at the start period of the exercise.
the noncontracting muscle cannot be neglected. Taylor et al. (50) reported that with the use of venous occlusion plethysmography, vascular resistance of the noncontracting leg was unchanged at the initial period (0–1.5 min) of one-legged cycling at an intensity of 38–75% of the peak O2 uptake and only increased during the later period (2–3 min) of the exercise, depending on the intensity. Yoshizawa et al. (53) reported using ultrasound Doppler flowmetry as an intensity-dependent increase in femoral vascular conductance of the nonexercising leg at the start of one-legged knee extension exercise at 15–45% of the maximal voluntary contraction. The initial increase in femoral vascular conductance was followed by a decrease in femoral vascular conductance at the end (~2–4 min) of 30–45% exercise but not 15% (53). Taking the previous and present findings into consideration, it is likely that intensity-dependent vasodilatation occurs in the nonexercising limb at the start of one-legged exercise and is probably mediated by either muscle mechanoreflex or central command, whereas vasoconstriction occurs in the nonexercising limb during the later period (>2 min) of one-legged exercise, in particular, at a higher intensity. This may be mediated by muscle metaboreflex, because posthandgrip ischemia caused vasoconstriction in the calf vascular bed (46).

Neurogenic Mechanism Responsible for the Vasodilatation in the Noncontracting Muscle

Either muscle mechanoreflex or central command is a possible neurogenic mechanism responsible for the initial hyperemia in the noncontracting muscle in this study, because muscle metaboreflex and β-adrenergic vasodilatation, via circulating catecholamines from adrenal medulla, are not sufficiently rapid to induce the initial hyperemia. In this study, we used passive one-legged cycling to isolate an influence of muscle mechanoreflex on muscle blood flow, although passive exercise cannot fully develop muscular tension as voluntary exercise and cannot always mimic the mechanical event during the voluntary exercise. No increase in Oxy-Hb of the noncontracting VL was detected at the start period of passive cycling, and subsequently, Oxy-Hb increased gradually in parallel with a decrease in TPR. This result suggests that muscle mechanoreflex does not account for the initial hyperemic response in the noncontracting VL, but it may induce delayed muscle vasodilatation.

Thus it is likely that central command contributes to increased tissue blood flow in the noncontracting muscle at the start of voluntary exercise. The two conventional ideas about a sympathetic mechanism responsible for centrally induced vasodilatation are sympathetic withdrawal and sympathetic cholinergic vasodilatation. Callister et al. (11) reported that muscle sympathetic nerve activity (MSNA) to a resting arm is inhibited during a period of preparation and initiation of ergometer exercise, suggesting sympathetic withdrawal prior to and at the start of voluntary exercise. However, the response of MSNA to the resting lower leg during one-legged cycling is controversial [MSNA increased (24), decreased (43), and unchanged (40)]. Fisher et al. (22) reported that vascular conductance in the resting leg transiently increased at the onset of contralateral isometric calf exercise, whereas MSNA to the resting lower leg was unchanged, suggesting that the transient increase in vascular conductance at the onset of exercise is unrelated to changes in MSNA. Therefore, it is unlikely that a decrease in MSNA causes the initial muscle vasodilatation. On the other hand, Sanders et al. (44) reported that the initial vasodilatation in the nonexercising limb was blocked by atropine but not by propranolol, suggesting that activation of sympathetic cholinergic vasodilator fibers induced neurogenic vasodilatation.

Taking the results of the human study and our previous finding of the cholinergic vasodilatation at the onset of voluntary static exercise in the cat (31) into consideration, it is possible that central command may induce the cholinergic vasodilatation in the noncontracting muscle at the start of voluntary exercise in humans. However, direct evidence for activation of sympathetic cholinergic fibers remains to be studied.

Bilateral Muscle Vasodilatation by Central Command

Motor imagery of exercise increases HR and respiratory rate (15), suggesting that central command involved in imagery of exercise can activate the cardiovascular and respiratory systems without muscular contraction. Thus an increase in skeletal muscle blood flow is expected, not only during voluntary exercise but also during motor imagery of the exercise. In fact, cycling-imagery increased femoral blood flow and Oxy-Hb with no changes in Deoxy-Hb and perfusion pressure (Figs. 6 and 7). This finding suggests a centrally induced muscle vasodilatation. Since the internal diameter of the femoral artery did not change during the motor imagery, the centrally induced vasodilatation should occur in peripheral resistance vessels. The result is supported by a previous finding that electrical stimulation of the hypothalamic defense area evoked large increases in the internal diameter and cross-sectional area of small arteries in the hindlimb triceps surae muscle in anesthetized cats (36). In contrast, circle-imagery with no relation to exercise induced no significant changes in femoral blood flow and Oxy-Hb, suggesting that imagery per se did not induce an increase in muscle blood flow. Since the VAS score was not significantly different between the cycling- and circle-imagery protocol, the extent of vividness involved during imagery could not explain the difference in the hyperemic response between cycling- and circle-imagery. Consequently, the activation of central neurons related to “motor” imagery is important for evoking neurogenic vasodilatation in muscle tissue.

Interestingly, cycling-imagery evoked similar increases in Oxy-Hb of the bilateral VL muscles (Fig. 7A). This result suggests that central command induced simultaneous neurogenic vasodilatation in the bilateral skeletal muscles to the same extent during imagery of exercise. Lee et al. (32) reported that many of the medullary neurons, especially in the rostral ventrolateral medulla and raphe nuclei, were dually infected by recombinants of pseudorabies virus injected into the bilateral hindlimbs of rats, suggesting that numerous sympathetic premotor neurons innervate both left and right hindlimb. Since voluntary exercise should be accompanied with central command, centrally induced vasodilator signals will be equally transmitted to the bilateral VL muscles, not only during imagery of exercise but also during voluntary exercise. If so, central command would induce bilateral vasodilatation in the contracting and noncontracting muscle to the same extent during voluntary one-legged exercise. Indeed, at the start of voluntary cycling, the Oxy-Hb in the contracting VL increased abruptly with the same time course and magnitude as the Oxy-Hb of
noncontracting VL, whereas Deoxy-Hb was unchanged in both muscles (Fig. 9). Thus, it is suggested that muscle tissue blood flow increased equally in both the contracting and noncontracting VL at the start of voluntary cycling.

In conclusion, we propose a novel concept that central command is likely to transmit the vasodilator signal to bilateral skeletal muscles, not only during motor imagery but also at the start of voluntary exercise in humans. Since daily physical activity, such as walking, is performed with both legs, centrally induced bilateral vasodilatation at the start of exercise may be reasonable for meeting the initial oxygen demand in skeletal muscles involved during exercise.

ACKNOWLEDGMENTS

We thank Ms. Tomoko Ishida for her excellent technical assistance. We also appreciate Dr. Hitoshi Okamura and the Center for Advanced Practice and Research of Rehabilitation for kindly lending us a near-infrared spectrometer and an EMG recording system.

GRANTS

Support for this study was provided by Grants-in-Aid for Scientific Research (B) and for exploratory research from the Japan Society for the Promotion of Science.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


