Noninvasive assessment of sympathetic vasoconstriction in human and rodent skeletal muscle using near-infrared spectroscopy and Doppler ultrasound

Paul J. Fadel,1 David M. Keller,2 Hitoshi Watanabe,1 Peter B. Raven,2 and Gail D. Thomas1

1Hypertension Division, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas 75390; and 2Department of Integrative Physiology, University of North Texas Health Science Center, Fort Worth, Texas 76107

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Fadel, Paul J., David M. Keller, Hitoshi Watanabe, Peter B. Raven, and Gail D. Thomas. Noninvasive assessment of sympathetic vasoconstriction in human and rodent skeletal muscle using near-infrared spectroscopy and Doppler ultrasound. J Appl Physiol 96: 1323–1330, 2004. First published December 2, 2003; 10.1152/japplphysiol.01041.2003.—The precise role of the sympathetic nervous system in the regulation of skeletal muscle blood flow during exercise has been challenging to define in humans, partly because of the limited techniques available for measuring blood flow in active muscle. Recent studies using near-infrared (NIR) spectroscopy to measure changes in tissue oxygenation have provided an alternative method to evaluate vasomotor responses in exercising muscle, but this approach has not been fully validated. In this study, we tested the hypothesis that sympathetic activation would evoke parallel changes in tissue oxygenation and blood flow in resting and exercising muscle. We simultaneously measured tissue oxygenation with NIR spectroscopy and blood flow with Doppler ultrasound in skeletal muscle of conscious humans (n = 13) and anesthetized rats (n = 9). In resting forearm of humans, reflex activation of sympathetic nerves with the use of lower body negative pressure produced graded decreases in tissue oxygenation and blood flow that were highly correlated (r = 0.80, P < 0.0001). Similarly, in resting hindlimb of rats, electrical stimulation of sympathetic nerves produced graded decreases in tissue oxygenation and blood flow velocity that were highly correlated (r = 0.93, P < 0.0001). During rhythmic muscle contraction, the decreases in tissue oxygenation and blood flow evoked by sympathetic activation were significantly attenuated (P < 0.05 vs. rest) but remained highly correlated in both humans (r = 0.80, P < 0.006) and rats (r = 0.92, P < 0.0001). These data indicate that, during steady-state metabolic conditions, changes in tissue oxygenation can be used to reliably assess sympathetic vasoconstriction in both resting and exercising skeletal muscle.

THE SYMPATHETIC NERVOUS SYSTEM plays an important role in the regulation of skeletal muscle vasomotor tone, both at rest and during exercise. Our understanding of the sympathetic neural control of muscle blood flow has been greatly enhanced by animal studies in which mainly invasive techniques such as perivascular Doppler ultrasound (6, 24, 28, 29), microsphere deposition (14, 20), and intravital microscopy (1, 31) have been used to measure blood flow or blood vessel diameter. In humans, however, such studies have been challenging to perform because many of the conventional methods used to measure blood flow (e.g., plethysmography, tracer dilution) are limited by poor temporal or spatial resolution, required technical expertise, invasive nature, and/or sensitivity to movement artifact during muscle contraction (23).

A viable alternative to the more traditional hemodynamic approaches for evaluating vascular responses in human skeletal muscle may be the use of near-infrared (NIR) spectroscopy to measure changes in muscle oxygenation (7, 12, 25). This technique exploits the principle that NIR light easily penetrates tissues where it is differentially absorbed by the oxygenated and deoxygenated forms of hemoglobin and myoglobin (21, 22). Changes in the absorption of NIR light are proportional to changes in the relative concentrations of these molecules, thereby providing a continuous noninvasive measure of the local balance between oxygen supply and demand. When oxygen demand is constant, changes in NIR absorption should mainly reflect changes in oxygen supply (i.e., blood flow). On the basis of this rationale, previous studies in humans showing that sympathetic activation decreased muscle oxygenation more in resting than in exercising forearm concluded that sympathetic vasoconstriction was attenuated during exercise (7, 12, 25). However, simultaneous measurements of forearm muscle oxygenation and blood flow have never been performed to fully validate this conclusion.

In this study, we hypothesized that sympathetic activation would elicit qualitatively similar changes in blood flow and tissue oxygenation in resting skeletal muscle that are attenuated in parallel in exercising muscle. To test this in humans, we simultaneously measured changes in brachial artery blood flow with Doppler ultrasound and forearm muscle oxygenation with NIR spectroscopy in response to reflex sympathetic activation evoked by lower body negative pressure (LBNP). We also performed complementary experiments in the hindlimb of anesthetized rats, using electrical stimulation of the paravertebral lumbar nerves to standardize and extend the range of sympathetic activation beyond that used in the human experiments.

METHODS

Human Subjects

Thirteen healthy volunteer subjects (8 men, 5 women) participated in the study. The mean age, height, and weight were 28 ± 2 yr, 174 ± 3 cm, and 72 ± 5 kg, respectively. All subjects were normotensive, had no history of cardiovascular disease, and were not taking any prescription medication. Each subject had no history of hypertension, diabetes mellitus, or dyslipidemia. This protocol was approved by the Human Subjects Committee at the University of Texas Southwestern Medical Center, and all subjects gave written informed consent.

Address for reprint requests and other correspondence: G. D. Thomas, Div. of Hypertension, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-8986 (E-mail: gail.thomas@utsouthwestern.edu).
medications. Informed, written consent was obtained from each subject. All protocols were approved by the University of Texas Southwestern Medical Center Institutional Review Board and were conducted in accordance with the Declaration of Helsinki principles. On experimental days, subjects were asked to abstain from caffeinated beverages for a minimum of 12 h and from strenuous physical activity for 24 h before the study.

Experimental measurements. Subjects were studied in the supine position. Heart rate was recorded continuously by electrocardiogram, and arterial blood pressure was measured by automated oscillometric sphygmanometry (model CE0050, Welch Allyn, Skaneateles Falls, NY). Maximal voluntary contraction (MVC) was determined as the greatest of at least three maximal squeezes of a handgrip dynamometer (Stoelting, Chicago, IL).

Forearm blood flow. Brachial artery mean blood flow velocity (MBV) was determined by using pulsed Doppler ultrasound velocimetry. Blood flow velocity spectra and arterial diameter were measured (Toshiba Sonolayer, model SSH-140A, Toshiba American Medical Systems, Tustin, CA) by using a 3.75-MHz pulsed Doppler probe placed on the skin over the brachial artery on the distal one-third of the upper arm at an insolation angle of 45°. All Doppler data were recorded onto VHS tape and analyzed with custom software (Toshiba, Toshiba American Medical Systems). Beat-to-beat MBV was computed from the area under the instantaneous velocity profile during each cardiac cycle. Brachial artery diameter was measured at the same site as MBV. Forearm blood flow was calculated as the product of MBV and arterial cross-sectional area. Forearm vascular conductance was calculated as the quotient of forearm blood flow and mean arterial pressure times 100 (units, ml min⁻¹ 100 mmHg⁻¹).

Forearm muscle oxygenation. Continuous-wavelength NIR spectroscopy was used to measure changes in the absorption of NIR light by oxygenated and deoxygenated hemoglobin plus myoglobin (HbO₂, MbO₂, and Hb, respectively). Individual contributions of hemoglobin and myoglobin to the NIR signal cannot be readily separated due to the presence of hemoglobin, which is normally confined to the vascular space (27). Moreover, in addition, changes in the NIR signals reflect changes in light absorption by hemoglobin in the small arterioles, capillaries, and venules of the microcirculation because large vessels (>1-mm diameter) contain sufficient hemoglobin to maximally absorb NIR light (16). Therefore, during steady-state conditions when oxygen utilization is constant, changes in the NIR signal should primarily reflect changes in oxygen delivery or blood flow at the level of the microcirculation.

To monitor the tissue absorption of NIR light, two fiber-optic bundles spaced 2 cm apart were placed directly on the skin over the left flexor digitorum profundus muscle, which is the main muscle recruited during handgrip (10). NIR signals at four different wavelengths (775, 825, 850, and 905 nm) were sequentially sampled at a rate of 1 Hz and converted to optical densities, which were output to a personal computer and stored digitally for later analysis. To calculate absolute concentrations from changes in optical density, the pathlength of light in the tissue must be known. This is, however, difficult to determine in vivo. Because absolute concentrations were not essential for the purposes of this study, we chose instead to express the changes in the NIR signals as a percentage of the maximal physiological range, or total labile signal (TLS). The TLS was determined in each experiment as the difference between baseline and complete deoxygenation during sustained circulatory arrest produced by inflating a pneumatic cuff on the upper arm to 220 mmHg.

LBNP. The subject’s lower body was enclosed in a negative pressure chamber to the level of the iliac crest. Pressure inside the chamber was measured continuously by a Statham transducer. Application of LBNP has been shown to cause highly reproducible reflex increases in muscle sympathetic nerve activity before, during, and after handgrip exercise (12, 26).

Experimental protocol. Arterial blood pressure, forearm blood flow, and forearm tissue oxygenation were measured during 2 min of LBNP at −10, −20, −30, or −40 mmHg applied in random order under resting conditions. A minimum of 10 min separated each LBNP trial. In some subjects, studies were conducted on 2 separate days to assess the reproducibility of the LBNP-induced changes in forearm blood flow and tissue oxygenation. Responses to LBNP at −20 mmHg were also evaluated during the third to fifth minute of a 6-min bout of rhythmic handgrip performed at 30% MVC. Subjects matched force production to a visual target, and a metronome provided auditory cues for each contraction and relaxation phase of the exercise (40 beats/min; 50% duty cycle).

Anesthetized Animals

The protocols used in this study were approved by the Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center.

Experimental preparation. Nine female Sprague-Dawley rats (232–297 g; Charles River, Kingston, MA) were anesthetized with ketamine (80 mg/kg ip) and α-chloralose (60 mg/kg iv supplemented with 10 mg·kg⁻¹·h⁻¹ iv). Atropine sulfate (0.5 mg/kg sc) was administered, the cervical trachea was cannulated, and the animals were ventilated with room air and supplemental oxygen. Rats were instrumented with jugular vein and carotid artery catheters. Stimulating electrodes were affixed to the left sciatic nerve to produce hindlimb contraction, and the left hindlimb was connected to a force-displacement transducer (model FT-10, Grass Instruments, Quincy, MA) via the calcaneal tendon. Core temperature was maintained at 37°C with an external heat source. Rats were allowed to stabilize for at least 20 min before protocols were begun. At the end of each experiment, rats were euthanized with pentobarbital sodium (150 mg/kg iv).

Femoral blood flow. A Doppler flow probe was placed around the left femoral artery to measure changes in blood flow velocity by recording the pulsatile and mean Doppler shifts in kilohertz with a VF-1 pulsed Doppler flow system (Crystal Biotech, Holliston, MA). Femoral vascular conductance was calculated online as the mean Doppler shift divided by mean arterial pressure times 100 (units, kHz/100 mmHg).

Hindlimb muscle oxygenation. With the use of the same NIR spectrophotometer as in the human experiments, two fiber-optic bundles spaced 1 cm apart were placed directly on the exposed lateral surface of the left gastrocnemius muscle. NIR signals were sequentially sampled at a rate of 1 Hz and converted to optical densities, which were output to a personal computer and digitally stored for later analysis. Changes in the NIR signals are expressed as a percentage of the TLS, which was defined in each experiment as the difference between baseline and complete deoxygenation observed during maximal hindlimb ischemia produced by lumbar sympathetic nerve stimulation at 20 Hz.

Lumbar sympathetic nerve stimulation. After an anterior abdominal incision, the left lumbar sympathetic chain was isolated inferior to the renal artery, placed on bipolar platinum electrodes, and covered with dental silicone (S4i, Bisico, Bielefeld, Germany).

Experimental protocol. Arterial blood pressure, femoral blood flow velocity, and tissue oxygenation were measured during 2 min of lumbar sympathetic nerve stimulation (LNS) by using 1-ms pulses of 5 V at 0.25, 0.50, 1.0, 1.5, 2.0, or 2.5 Hz performed in random order in resting hindlimb. LNS at 1.0 and 2.5 Hz were repeated during intermittent tetanic contractions of the hindlimb at 33% of maximal
tension produced by stimulation of the sciatic nerve with 250-ms trains of pulses (100 Hz, 0.2-ms) at a rate of 1 Hz.

Data Analysis and Statistics

Hemodynamic and cardiovascular data were acquired and analyzed by using PowerLab hardware and software (ADInstruments, Milford, MA). For both the human and animal studies, all variables were assessed over a period of 20 s. Sympathetic responses to LBNP or LNS were determined by calculating the difference between the mean of 20 s of baseline data immediately preceding the sympathetic stimulus and the mean of the last 20 s of data during the stimulus. In the present study, sympathetic activation evoked reciprocal changes in HbO₂/MbO₂ and Hb/Mb as our laboratory has previously reported (12). We have chosen to present only the HbO₂/MbO₂ data in this paper because it is intuitively easier to relate sympathetically mediated decreases in blood flow with decreases in HbO₂/MbO₂, rather than with increases in Hb+Mb. Statistical analysis was performed by using single-factor repeated-measures ANOVA followed by Bonferroni post hoc tests. Correlation coefficients between tissue oxygenation and blood flow or vascular conductance were derived by using multiple regression models to partition the variation due to subjects (2). In the human subjects, day-to-day repeatability of the tissue oxygenation and blood flow responses to LBNP was assessed by using Bland-Altman comparison methods of agreement (3). P < 0.05 was considered significant. Data are presented as means ± SE.

RESULTS

In Humans, Reflex Sympathetic Activation Evokes Parallel Changes in Tissue Oxygenation and Blood Flow in Resting and Exercising Forearm

Baseline hemodynamics acquired before each level of LBNP were not significantly different. Average baseline values for mean arterial pressure, heart rate, forearm blood flow, and forearm vascular conductance were 81 ± 1 mmHg, 61 ± 1

Table 1. Responses to lower body negative pressure in human subjects at rest

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<th>Lower Body Negative Pressure</th>
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<th>-30 mmHg</th>
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<tr>
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<td>-17±1*</td>
<td>-23±2*</td>
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Values are means ± SE. ΔMAP, change in mean arterial pressure; ΔHR, change in heart rate; ΔHbO₂+MbO₂, change in oxygenated hemoglobin plus oxygenated myoglobin; TLS, total labile signal. *Significant response, P < 0.05.

Fig. 1. A and B: original records showing tissue oxygenation [oxygenated hemoglobin plus oxygenated myoglobin (HbO₂+MbO₂)] and beat-to-beat arterial blood velocity waveforms in resting human forearm in response to graded reflex sympathetic activation evoked by lower body negative pressure (LBNP). The total labile signal (TLS) was acquired at the end of the experiment as described in the text. C: summary data (means ± SE; n = 13 subjects) showing the relationship between sympathetically mediated changes in tissue oxygenation and forearm blood flow (top) or forearm vascular conductance (bottom). OD, optical density; Δ, change.
correlated with the decreases in blood oxygenation evoked by LBNP at during both rest and exercise, the decreases in forearm muscle oxygenation (HbO2) and vascular conductance (r = 0.93, 95% confidence interval: 0.91-0.94, P < 0.0001) and vascular conductance (r = 0.95, P < 0.0001).

In resting hindlimb, LNS produced frequency-dependent decreases in tissue oxygenation, blood flow velocity, and vascular conductance (Table 2, Fig. 4). The sympathetically mediated decreases in tissue oxygenation were highly correlated with the reductions in blood flow velocity (r = 0.93, P < 0.0001) and vascular conductance (r = 0.95, P < 0.0001).

Intermittent tetanic contraction of the hindlimb elicited increases in heart rate (+5 ± 6 beats/min; P = not significant (NS)), mean arterial pressure (+6 ± 3 mmHg; P = NS), and blood flow velocity (+1.63 ± 0.22 kHz; P < 0.05), and it elicited decreases in tissue oxygenation (−6 ± 9%; P = NS). Compared with responses to 1-Hz LNS in resting hindlimb, contraction attenuated the decreases in tissue oxygenation (−27 ± 3 vs. −1 ± 3%; P < 0.05), blood flow (−16 ± 2 vs. +2 ± 3%; P < 0.05), and vascular conductance (−27 ± 2 vs. −9 ± 2%; P < 0.05). Responses to 2.5-Hz LNS also were attenuated in contracting hindlimb: tissue oxygenation (−66 ± 6 vs. −15 ± 3%; P < 0.05), blood flow (−28 ± 3 vs. −9 ± 2%; P < 0.05), and vascular conductance (−47 ± 4 vs. −15 ± 2%; P < 0.05). Similar

Baseline hemodynamics acquired before each level of LNS were not significantly different. Average baseline values for mean arterial pressure, femoral blood flow velocity, and femoral vascular conductance were 88 ± 2 mmHg, 1.47 ± 0.09 kHz, and 1.68 ± 0.10 kHz/100 mmHg, respectively.

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to the results of the human protocol, the changes in oxygenation in resting and contracting muscle evoked by 1- or 2.5-Hz LNS were strongly correlated with changes in blood flow velocity and vascular conductance (Fig. 5).

DISCUSSION

Understanding the role of the sympathetic nervous system in the regulation of skeletal muscle blood flow during exercise has proven to be particularly challenging in humans, in part because of limitations associated with conventional techniques used to measure blood flow in active muscle (23). NIR spectroscopy provides an attractive noninvasive method to evaluate vasomotor responses in exercising muscle because of its high spatial and temporal resolution, providing continuous measurements of oxygen availability at the level of the microcirculation (21, 22). In the present study, our complementary experiments in human and rat skeletal muscle indicate that a given vasoconstrictor stimulus evokes highly correlated decreases in tissue oxygenation measured by NIR spectroscopy and blood flow measured by Doppler ultrasound. These new data demonstrate that, during steady-state metabolic conditions, changes in tissue oxygenation can be used to reliably assess sympathetically mediated vasoconstriction in both resting and exercising muscle.

Although the correlation coefficients relating changes in tissue oxygenation to blood flow were highly significant in all of our experiments, correlations derived from the rat experiments were consistently stronger than those from the human experiments. This likely reflects several differences in the experimental design between the animal and human studies. First, the vasoconstrictor stimulus was much more uniform in the animal experiments that used direct electrical stimulation of sympathetic nerves compared with reflex activation in the human experiments. Second, in the animal experiments, the placement of the NIR optodes directly on the exposed muscle and the Doppler flow probe directly on the artery likely increased the agreement between these two measures. In contrast, in the human subjects, both the optical and acoustic
signals were subject to additional absorption and scattering in the skin and subcutaneous adipose tissue. Third, the tissue volume sampled by NIR spectroscopy in the animal experiments was a large fraction of the total muscle volume because the optodes nearly covered the entire lateral surface of the gastrocnemius muscle. In contrast, in the human experiments, these same optodes sampled a relatively smaller volume of the flexor digitorum muscle of the forearm.

An important feature of our human studies was the demonstration that reflex sympathetic activation by LBNP evoked similar decreases in forearm muscle oxygenation and blood flow in a subset of subjects tested on 2 separate days. Although we did not measure the sympathetic vasoconstrictor stimulus in this study, LBNP-induced activation of muscle sympathetic nerves is known to be highly reproducible within individual subjects (12, 26). We now extend these findings to show that NIR and Doppler responses to a given level of LBNP also are very reproducible, rendering them useful for longitudinal studies comparing vasomotor responses before and after a particular intervention (e.g., exercise training or drug treatment).

On the basis of the qualitatively similar decreases in tissue oxygenation and blood flow in response to sympathetic activation, we have concluded that changes in tissue oxygenation can be used to assess sympathetic vasoconstriction in skeletal muscle. However, the NIR measurements are sensitive to changes in blood volume as well as blood flow, raising the possibility that the sympathetically mediated decreases in tissue oxygenation primarily reflect decreases in venous volume rather than arterial flow. We believe that this is unlikely for several reasons. First, both histochemical and functional evidence indicates that in the muscle microcirculation sympathetic innervation of arterioles is dense but that of venules is sparse or nonexistent (11, 17, 19). Because NIR spectroscopy measures changes in oxygenation mainly in the microcirculation (16), the decreases in the NIR signals observed during sympathetic activation should primarily reflect arteriolar, not venular, constriction. Second, we have measured tissue oxygenation responses to LBNP in human forearm when the arm is positioned at heart level or elevated above heart level to produce venous drainage. This maneuver did not affect the LBNP-induced decrease in tissue oxygenation (P. J. Fadel and G. D. Thomas, unpublished observations), and, therefore, we suggest that changes in venous volume do not significantly contribute to the observed vasoconstrictor responses.

During muscle contraction, the sympathetically mediated changes in tissue oxygenation and muscle blood flow remained highly correlated but were attenuated compared with responses in resting muscle in both humans and rats. In the animal experiments, the degree of attenuation varied inversely with the strength of the vasoconstrictor stimulus as our laboratory and others previously reported (9, 29), but there was slightly less attenuation of the vasoconstrictor response to a given sympathetic stimulus in the present study compared with our laboratory’s previous rat studies (9, 28, 29). This probably was due to differences in experimental protocols because in the present study the rat hindlimb was contracted at a submaximal

Fig. 5. A: relationship between changes in tissue oxygenation (HbO₂ + MbO₂) and femoral blood flow velocity evoked by 1-Hz (left) or 2.5-Hz (right) lumbar nerve stimulation (LNS) performed before, during, and after hindlimb contraction in 8 rats. B: relationship between changes in tissue oxygenation and femoral vascular conductance evoked by 1-Hz (left) or 2.5-Hz (right) LNS performed before, during, and after hindlimb contraction.
nonfatiguing intensity (33% of maximum) to more closely mimic our human experiments (30% MVC), whereas maximal fatiguing contractions were used in the previous rat studies. Exercise intensity is one of the factors reported to modulate sympathetic vasoconstriction in active muscle (6, 12, 24, 28, 30).

The results of our human studies showing that the decreases in tissue oxygenation and blood flow evoked by reflex sympathetic activation in resting forearm were attenuated by ~75% during rhythmic handgrip are consistent with recent human studies from two other laboratories. Using an experimental protocol similar to ours, Tschakovsky et al. (30) reported that decreases in blood flow (measured by Doppler ultrasound) evoked by tyramine-induced release of endogenous norepinephrine from sympathetic nerve terminals in resting forearm also were attenuated by ~75% during rhythmic handgrip. Ogata et al. (18) reported that oxygenation (measured by NIR spectroscopy) decreased in resting leg muscle when arm exercise was used to reflexively activate sympathetic nerves. In contrast, arm exercise did not decrease oxygenation in the leg muscles during cycling exercise. Taken together, these studies provide increasing evidence that the modulation of sympathetic vasoconstriction in exercising human skeletal muscle can be readily detected by ultrasound measurements of blood flow or spectroscopic measurements of tissue oxygenation.

Careful consideration must be given to the experimental conditions when using NIR measurements of tissue oxygenation to assess vascular responses. Although our data show that, under steady-state metabolic conditions a given sympathetic vasoconstrictor stimulus evokes highly correlated decreases in tissue oxygenation and blood flow, this relationship may not be maintained under all conditions. For example, tissue oxygenation and muscle blood flow likely are dissociated at the onset of dynamic exercise when both oxygen demand and delivery are increasing, just as venous oxygen saturation and muscle blood flow are dissociated (15). Under such non-steady-state conditions, NIR spectroscopy could be used in combination with venous occlusion (8, 13) or the light-absorbing tracer indocyanine green (4) to directly assess blood flow. The advantages and limitations of using NIR spectroscopy to quantify absolute blood flow measurements have recently been described in detail (4, 5).

In summary, our complementary human and rat experiments indicate that tissue oxygenation responses can be used to reliably evaluate sympathetic vasoconstriction in active and inactive skeletal muscle under appropriately controlled conditions. In light of the limited techniques available for measuring vasomotor function in exercising muscle in humans, this study demonstrates the utility of NIR spectroscopy as a simple, noninvasive tool to indirectly assess relative changes in local blood flow evoked by sympathetic activation. The high spatial and temporal resolution of NIR spectroscopy coupled with the relative stability of the optode placement might make it particularly well suited for studying 1) sympathetically mediated responses in individual small muscles in a larger group of exercising muscles, 2) the heterogeneity of sympathetic responses within larger muscles, or 3) sympathetically mediated responses during high-intensity exercise. On the other hand, a technique such as Doppler ultrasound might be preferred over NIR spectroscopy when whole limb or absolute blood flow measurements are required or when measurements are performed during non-steady-state metabolic conditions (e.g., fatiguing exercise).

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