Skin blood flow influences near-infrared spectroscopy-derived measurements of tissue oxygenation during heat stress

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Davis, Scott L., Paul J. Fadel, Jian Cui, Gail D. Thomas, and Craig G. Crandall. Skin blood flow influences near-infrared spectroscopy-derived measurements of tissue oxygenation during heat stress. J Appl Physiol 100: 221–224, 2006. First published September 8, 2005; doi:10.1152/japplphysiol.00867.2005.—Near-infrared (NIR) spectroscopy is a noninvasive optical technique that is increasingly being used to assess muscle oxygenation during exercise with the assumption that the contribution of skin blood flow to the NIR signal is minimal. We tested this assumption in humans by monitoring forearm tissue oxygenation during selective cutaneous vasodilation induced by locally applied heat (n = 6) or indirect whole body heating (i.e., heating subject but not area surrounding NIR probes; n = 8). Neither perturbation has been shown to cause a measurable change in muscle blood flow or metabolism. Local heating (~41°C) caused large increases in the NIR-derived tissue oxygenation signal [before heating = 0.82 ± 0.89 optical density (OD), after heating = 18.21 ± 2.44 OD; P < 0.001]. Similarly, whole body heating (increase internal temperature 0.9°C) also caused large increases in the tissue oxygenation signal [before heating = −0.31 ± 1.47 OD, after heating = 12.48 ± 1.82 OD; P < 0.001]. These increases in the tissue oxygenation signal were closely correlated with increases in skin blood flow during both local heating (mean r = 0.95 ± 0.02) and whole body heating (mean r = 0.89 ± 0.04). These data suggest that the contribution of skin blood flow to NIR measurements of tissue oxygenation can be significant, potentially confounding interpretation of the NIR-derived signal during conditions where both skin and muscle blood flows are elevated concomitantly (e.g., high-intensity and/or prolonged exercise).

Near-infrared (NIR) spectroscopy is a noninvasive optical technique that is increasingly being used to assess changes in tissue oxygenation during exercise (8, 16). This technique is based on the principle that NIR light easily penetrates biological tissues, such as muscle, adipose, and skin (20), where it is absorbed by the iron or copper centers of hemoglobin, myoglobin, and mitochondrial cytochrome-c oxidase. Under normal conditions, the majority of NIR light absorption is due to the presence of hemoglobin in the small arterioles, capillaries, and venules of the microcirculation (16). These features have led to growing interest in the use of NIR spectroscopy to monitor local changes in muscle oxygenation and blood flow during both submaximal and maximal exercise (1, 2, 19, 25). However, NIR light applied to the surface of the body must first pass through the overlying skin and subcutaneous adipose layers before it reaches muscle. For this reason, the potential impact of these superficial tissues on NIR light absorption and scattering should be considered when using NIR spectroscopy to assess muscle oxygenation (3, 24).

Because skin blood flow can increase markedly as core temperature increases, the effect of cutaneous vasodilation on the NIR-derived measures of tissue oxygenation is of particular concern during exercise. In previous NIR spectroscopic studies in which subjects performed brief exercise of small muscle masses (e.g., handgrip), skin blood flow likely remained low and had minimal influence on tissue oxygenation (4, 8, 12). However, NIR spectroscopic studies are increasingly being conducted in subjects performing prolonged, dynamic exercise of large muscle masses (5, 10, 13, 18, 19, 26). Such exercise protocols can elicit pronounced increases in skin blood flow that could potentially influence NIR light absorption and scattering. Although prior studies in humans concluded that skin blood flow contributed minimally to NIR-derived tissue oxygenation, these studies were limited by the small sample sizes and by the methodologies used to increase and assess skin blood flow (11, 16).

Therefore, the purpose of this investigation was to test the hypothesis that increases in skin blood flow during local heating and whole body heating, independent of changes in muscle blood flow, significantly influence the noninvasive measurement of tissue oxygenation using NIR spectroscopy.

METHODS

Protocol 1: Local Heating

Human subjects. Six healthy individuals (5 men, 1 woman) participated in protocol 1. The mean age, height, and weight were 29 ± 4 yr, 174 ± 5 cm, and 69.9 ± 6.8 kg, respectively. Participants provided informed, written consent. All protocols were approved by the Institutional Review Board at the University of Texas Southwestern Medical Center at Dallas and Presbyterian Hospital of Dallas and were conducted in accordance with the Declaration of Helsinki principles. All subjects refrained from caffeine, alcohol, and exercise for 24 h before the study.

Instrumentation. Tissue oxygenation was assessed with continuous-wavelength NIR spectroscopy (model NIRO 500, Hamamatsu Photonics, Hamamatsu, Japan) as previously described (8). Two fiber-optic bundles spaced 2 cm apart were placed directly on the skin over the left flexor digitorum profundus muscle. The 2-cm distance between NIR optic bundles has been previously shown to optimize the NIR signal-to-noise ratio in the forearm (8, 9). 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 (OD) according to established algorithms. Skin blood flow was measured

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from an integrating laser-Doppler flowmetry probe (Perimed, Stockholm, Sweden) placed on the dorsal aspect (opposite side) of the same forearm. Laser-Doppler flowmetry measurements are specific to skin blood flow and are not influenced by underlying skeletal muscle blood flow (22). Skin temperature was measured via thermocouples attached to the skin at the location of the NIR optical bundles and laser-Doppler probe.

Protocol. After instrumentation, the forearm, including NIR optic bundles and laser-Doppler flow probes, was wrapped in a moist temperature-controlled heating pad. After 15 min of baseline data collection, skin temperature under the device was increased from bundles and laser-Doppler flow probes, was wrapped in a moist

Protocol 2: Whole Body Heating

Human subjects. Eight healthy individuals (5 men, 3 women) participated in protocol 2. The mean age, height, and weight were 33 ± 3 yr, 172 ± 5 cm, and 73.1 ± 6.2 kg, respectively.

Instrumentation. Tissue oxygenation was determined from continuous-wavelength NIR spectroscopy (model NlRO 300, Hamamatsu Photonics). Fiber spacing and sample wavelengths were similar to those used in protocol 1. Skin blood flow was measured via laser-Doppler flowmetry. However, in this protocol, the probe was placed adjacent to the NIR fiber-optic bundles over the left flexor digitorum profundus muscle. Internal temperature was measured from a thermometer placed in the sublingual sulcus. Mean skin temperature was measured via the weighted average of six thermocouples attached to the skin.

Protocol. Individuals were dressed in a tube-lined suit that permitted the control of skin temperature by changing the temperature of water perfusing the suit. The perfusion suit covered the entire body with the exception of the head, instrumented forearm, hands, and feet. Baseline measurements were recorded while perfusing the suit with 34°C water. After baseline data collection, a whole body heat stress ensued by perfusing 46°C water through the water perfusion suit until internal temperature increased ~0.8°C. For this protocol, the TLS was calculated from the peak NIR signal during whole body heating to the nadir of the signal during sustained suprasystolic cuff occlusion as described above.

Measurements and Statistical Analysis

For both protocols, data were continuously acquired at a sampling rate of 50 Hz using a data collection system (Biopac System, Santa Barbara, CA). Tissue oxygenation is expressed both as OD and as a percentage of the TLS. Due to differences in analog output between the NlRO 500 and the NlRO 300 spectrophotometers, the tissue oxygenation signal from protocol 1 (NlRO 500) was multiplied by a factor of 100. This linear adjustment resulted in an analog output that was similar between devices, thereby facilitating comparisons between protocols. Skin blood flow is reported in arbitrary units (AU). Mean values were calculated from 30-s averages at each stage during local heating and throughout whole body heating. Data points during whole body heating are expressed as a percentage of time to maximal heating (i.e., 25, 50, and 75% of the time needed to reach final internal temperature). Statistical analysis was performed by using an one-way repeated-measures ANOVA with Bonferroni post hoc tests if a significant main effect was observed. Statistical significance was accepted at \( P \leq 0.05 \). Linear regression analysis was performed separately on individual data to determine the relationship between tissue oxygenation and skin blood flow measurements during local heating and whole body heating. Correlation coefficients were calculated for each individual in both protocols. All data are presented as means ± SE.

RESULTS

Protocol 1: Local Heating

Local heating significantly increased tissue oxygenation (0.82 ± 0.89 to 18.21 ± 2.44 OD; \( P < 0.001 \)) and skin blood flow (26.0 ± 5.1 to 175.0 ± 20.0 AU; \( P < 0.001 \)) from baseline to peak local heating, respectively (Figs. 1 and 2). The TLS derived from the highest observed OD during local heating to the lowest observed OD during circulatory occlusion was 50.45 ± 4.37 OD. Local heating at ~37, ~39, and ~41°C increased tissue oxygenation by 5.19 ± 1.15, 13.73 ± 2.05, and 17.40 ± 1.82 OD, respectively. These increases in tissue oxygenation represent 10 ± 2, 25 ± 3, and 33 ± 2% of the TLS. Increases in tissue oxygenation and skin blood flow were closely correlated during local heating (mean \( r = 0.95 ± 0.02 \)).

Protocol 2: Whole Body Heating

Whole body heating increased skin temperature from 34.5 ± 0.2 to 37.9 ± 0.2°C, resulting in an increase in internal temperature from 36.3 ± 0.4 to 37.2 ± 0.4°C (\( P < 0.001 \)). Whole body heat stress significantly increased tissue oxygenation (~0.31 ± 1.37 to 12.48 ± 1.82 OD; \( P < 0.001 \)) and skin blood flow (33.6 ± 6.8 to 135.9 ± 13.2 AU; \( P < 0.001 \)) from baseline to maximal heating, respectively (Figs. 3 and 4). The
increased skin blood flow two- to fourfold, the magnitude of the thermal stress is unclear because neither water temperature, skin temperature, nor immersion time was reported (16). Furthermore, instrumentation for tissue oxygenation and skin blood flow measurements was delayed until after the subject’s forearm was removed from the water bath. Depending on the time needed for placement of the NIR optic bundles and laser-Doppler probe, skin blood flow (and perhaps tissue oxygenation) may have already undergone a substantial decrease before the first measurements were made.

The protocols used in the present study provided two distinct advantages for assessing the contribution of skin blood flow to NIR-derived measures of tissue oxygenation. First, the simultaneous, continuous measurement of tissue oxygenation and skin blood flow during well-controlled thermal stimuli provided reliable quantitative results that could be statistically analyzed. Second, it is well established that both local and whole body heating increase skin blood flow without changing muscle blood flow or metabolism (6, 7, 14, 15, 17, 21). Therefore, the increases in tissue oxygenation observed with either heating stimuli can be attributed solely to increases in skin blood flow with minimal, if any, contribution of muscle blood flow (7, 14, 15, 17, 21). Importantly, changes in NIR absorption that occurred during local heating of the forearm in protocol 1 of the present study were not due to a direct effect of heating the NIR optic bundles because similar results were obtained during whole body heating (protocol 2) in which the fiber-optic bundles were not in direct contact with the water perfusion suit. Consistent with this conclusion are the strong correlation coefficients of the relationship between the increase in tissue oxygenation and the increase in skin blood flow for both protocols.

On the basis of the present findings, investigators must carefully consider the contribution of skin to NIR-derived measurements of tissue oxygenation. Clearly, this concern is greatest for experimental protocols involving prolonged, dynamic exercise of large muscle masses that have the potential to cause a significant thermal stress, leading to large increases in skin blood flow. Although data from the present study were obtained from a relatively small muscle in the forearm, we believe that these data can be extrapolated to larger muscles (i.e., thigh). Previous research indicated that forearm and thigh cutaneous vasculatures dilate to similar levels during passive

**DISCUSSION**

The major new finding from the present investigation is that skin blood flow can contribute significantly to NIR-derived measurements of tissue oxygenation in humans. Using two distinct methods to increase skin blood flow (i.e., local and whole body heating), independent of changes in muscle blood flow, we found that elevations in skin blood flow caused parallel increases in tissue oxygenation. These data suggest that alterations in skin blood flow can influence NIR measurements of tissue oxygenation, potentially confounding interpretation of the NIR-derived signal during conditions where both skin blood flow and muscle blood flow are elevated concomitantly (e.g., high-intensity and/or prolonged exercise).

NIR spectroscopy provides an attractive noninvasive method to evaluate tissue oxygenation and blood flow responses in exercising muscle because of its high spatial and temporal resolution. Previously, the influence of skin blood flow on NIR-derived measurements of tissue oxygenation in humans was reported to be minimal (11, 16). The reasons for these conflicting results compared with the present study remain unclear but are likely due to differences in the protocols used to increase skin blood flow, as well as the small number of subjects investigated (i.e., 3–5). In the study by Hampson and Piantadosi (11), NIR-derived tissue oxygenation of a double-thickness skin remained unchanged during forearm ischemia and postischemic reactive hyperemia. Because skin blood flow was not measured in that study, the magnitude of change in flow during either ischemia or reactive hyperemia is not known. In the study by Mancini et al. (16), forearm tissue oxygenation measured by NIR spectroscopy did not change during recovery of skin blood flow from a local heat stress induced by forearm immersion in hot water. Although the authors stated that this local heating stimulus should have

![Fig. 3. Representative tracing of tissue oxygenation and skin blood flow responses during the whole body heating protocol (protocol 2). Symbols represent 1-min averages.](image)

![Fig. 4. Average tissue oxygenation and skin blood flow responses during whole body heating. Data points are expressed as a percentage of time to maximal heating. Values are means ± SE.](image)
heating (23). Therefore, increases in skin blood flow at the thigh should be a legitimate concern, with respect to using this site for NIR measurements, during a thermal stress or during exercise conditions in which internal temperature and skin temperature are elevated. Another important point for consideration is that the effect of skin blood flow on NIR light absorption also may be influenced by differences in NIR spectroscopy equipment, because the selection and number of wavelengths of light and the algorithms used to determine the absorption spectra vary from one instrument to another. Finally, the positioning and spacing of the NIR optic bundles may also affect the contribution of skin, because these factors determine the depth of light penetration into the tissues and the total tissue sampling volume.

In conclusion, increases in skin blood flow can have a significant impact on NIR-derived measurements of tissue oxygenation. NIR spectroscopy is increasingly being used to assess muscle oxygenation and blood flow in subjects performing prolonged, dynamic exercise of large muscle masses (5, 10, 13, 18, 19, 26). Depending on the workload and duration of these protocols, significant increases in skin blood flow could occur that, according to the present findings, would affect the NIR signal independent of changes in muscle oxygenation and muscle blood flow. Therefore, careful consideration must be given to the experimental conditions when interpreting NIR measurements of tissue oxygenation. Even small increases in skin blood flow may affect NIR light absorption and potentially confound the interpretation of the underlying muscle oxygenation measurement.

NOTE ADDED IN PROOF

Relevant information regarding the use of two different spectrophotometer models (Hamamatsu NIRO 300 and NIRO 500) that was unintentionally omitted from the METHODS of the accepted manuscript has been included in this final version.

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