Regional Differences of Hemodynamics and Oxygenation in the Human Calf Muscle Detected with Near-Infrared Spectrophotometry

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PURPOSE: Measurements in muscle tissue are often performed at a selected single location over the muscle of interest. The hypothesis is that the values obtained reflect the status within the entire muscle or muscle group. This, however, may not be the case. The study was performed to investigate whether this hypothesis is true for hemodynamics and oxygenation in the healthy human calf muscle at rest.

MATERIALS AND METHODS: Hemoglobin flow, blood flow, oxygen consumption, and venous hemoglobin oxygen saturation were mapped at 22 locations in 30 legs of 15 healthy subjects (nine women, six men aged 26–37 years) simultaneously by using frequency-domain near-infrared spectrophotometry with a specially designed probe during venous occlusion.

RESULTS: For all parameters, spatial heterogeneity was found between subjects and within individual legs. All parameters were highly significantly different when comparing proximal and distal regions. Differences were also found between medial and lateral regions. The global mean values (± standard deviation) over all measurements were as follows: hemoglobin flow, 1.27 µmol per 100 mL/min ± 0.88; blood flow, 0.56 mL per 100 g/min ± 0.38; oxygen consumption, 0.016 mL per 100 g/min ± 0.011; and venous oxygen saturation, 77.6% ± 5.9. The thickness of the overlying adipose tissue had an influence on the measurements and must be considered.

CONCLUSION: Highly significant spatial heterogeneity of hemodynamics and oxygenation was found in the healthy human calf muscle.

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Abbreviations: ATT = adipose tissue thickness, DPF = differential pathlength factor, NIRS = near-infrared spectrophotometry, $SvO_2$ = venous oxygen saturation, TRV = test-retest variability, $VO_2$ = oxygen consumption

NEAR-INFRARED spectrophotometry (NIRS) is a valuable tool for measuring hemodynamics and oxygenation in human tissue. NIRS is noninvasive, provides real-time measurements, is cost-effective, is feasible at the bedside, and is easily repeatable and reproducible and, therefore, suitable for use in the clinical setting. Because NIRS provides measurements at the level of arterioles, capillaries, and venules (1) several centimeters deep in the tissue, it is a complementary method to Doppler, which probes larger blood vessels, laser Doppler flowmetry, which measures superficial blood vessels, and imaging techniques such as magnetic resonance (MR) imaging, positron emission tomography (PET), and radiography, which depend on the use of ionizing radiation and/or contrast media. NIRS has been applied to infants and adults to measure hemodynamics and oxygenation in many fields such as the brain, but one particularly promising field is the skeletal muscle (2–5). Studies in the skeletal muscle have included measurements in sports medicine (6,7) and in patients with peripheral vascular disease (8–17), myopathies (18–20), and heart failure (21–23).

Measurements in muscle tissue are often performed at a selected single location over the muscle of interest. The values obtained are considered to reflect the status within the entire muscle or muscle group. However, hemodynamics and oxygenation may not be homogeneously distributed within the muscle tissue. Previous
studies showed spatial heterogeneity of blood flow in the human quadriceps muscle at rest by using PET and mean tissue oxygen saturation in the resting vastus lateralis by using NIRS (24,25). Patients with peripheral vascular disease also showed considerable spatial heterogeneity (15).

The purpose of this study was to evaluate the spatial distribution not only of blood flow as in previous studies (24) but also of hemoglobin flow, venous oxygen saturation (SvO₂), and oxygen consumption (VO₂) in the human calf muscle tissue of healthy subjects at rest.

MATERIAL AND METHODS

Subjects

Fifteen subjects, nine women and six men aged 26–37 years, participated in the study. Both calves of each subject were measured (30 legs). The inclusion criteria were that subjects had to be healthy and between the ages of 20 and 40 years. Written informed consent was obtained from all subjects before the experiments. This study was approved by the institutional review board of our university (IRB #94125).

Methods

NIRS is based on the principle that near-infrared light propagates through living tissue and that the attenuated light emerging from the tissue contains information about the optical properties of the tissue. The attenuation of light is caused by absorption and scattering. The latter increases the effective pathlength of the light and prevails in media such as the human tissue. NIRS exploits the fact that two major tissue components, oxyhemoglobin and deoxyhemoglobin, have absorption maxima at two different wavelengths in the near-infrared region and can therefore be differentiated by using light of different wavelengths. Concentration changes in oxyhemoglobin and deoxyhemoglobin can be determined from changes in absorption at different wavelengths. To correct for light scattering, the attenuation values must be divided by a correction factor, the differential pathlength factor (DPF), to quantify correctly the concentration values. The DPF, which varies depending on the tissue, has been determined for different types of tissue (26,27). However, DPF values for a discrete tissue vary in the literature, presumably because tissue composition may differ. Most NIRS instruments measure attenuation only and, therefore, depend on DPF values taken from the literature. One of the advantages of our frequency-domain instrument and sensor is that we can not only measure attenuation but can also differentiate between absorption and scattering and, therefore, determine the DPF.

Instrument.—We used a frequency-domain spectrophotometer (Oxy-Imager; ISS, Champaign, Ill). A detailed description of the frequency-domain technique can be found elsewhere (15,28). Briefly, the instrument operates at two wavelengths, 758 and 830 nm, which correspond to the absorption maxima of deoxyhemoglobin and oxyhemoglobin, respectively. The light generated by 32 laser diodes (16 per wavelength) is intensity-modulated at a frequency of 110 MHz. The light from the instrument to the tissue and back is guided through optical fibers. The light is collected in four photomultiplier tube detectors and demodulated. Then, its mean intensity, modulation amplitude, and phase are determined and stored in a computer. Maps over the entire region covered by the sensor were generated at a sample rate of 6 Hz.

Sensor.—The sensor covers an area of 18.5 × 6 cm (Fig 1) and enables us to measure locations simultaneously. The four detector fibers are equidistantly located along the middle line of the sensor. The source fibers are arranged at three different distances around the detector fibers. In the center of the sensor, the source detector distances are 2.4 cm and 3.5 cm. The symmetrical geometry in the shape of a parallelogram provides three center locations with a multidistance approach to determine absolute values for absorption and scattering, from which the DPF can be calculated (29). The distance between a detector and the other sources is 3.0 cm.

Measurement of Adipose Tissue Thickness (ATT).—Immediately after the NIRS measurement, the ATT of each leg was measured with a skinfold caliper (Lange, Beta Technology, Santa Cruz, CA) at nine locations over the probed area, that is, at three locations (medial, intermediate, lateral) per level (proximal, intermediate, distal). The measurement at each location was repeated three times and the mean value determined. This value was divided by a factor of 2 to obtain the ATT. The error of measurement was
less than 10%. The skinfold caliper method was chosen because it is relatively easy to apply, is portable, and the accuracy of its measurements is comparable to that with ultrasonography and MR imaging (30).

Protocol.—This study involved a venous occlusion, that is, a temporary stop of venous outflow. In the beginning of the occlusion, blood flows into the tissue at the same rate as that before the occlusion. The increase in total hemoglobin concentration thus reflects blood flow. Only a small amount of deoxyhemoglobin, accounted for by the arterial oxygen saturation, flows into the tissue through the arteries. Most of the increase in deoxyhemoglobin concentration during the venous occlusion stems from oxyhemoglobin, which is converted to deoxyhemoglobin by the VO$_2$ of the tissue. Thus, the increase in deoxyhemoglobin reflects oxygen consumption. There are several reasons for applying a venous occlusion. In combination with NIRS, it provides absolute quantization of physiologically important parameters such as the blood flow, tissue oxygenation, and oxygen consumption. Without venous occlusion or as an arterial occlusion, fewer parameters would be available. In addition, a venous occlusion causes no pain or discomfort and can thus be applied in patients with circulatory diseases.

Subjects lay in a supine comfortable position with both legs elevated slightly above the level of the heart to provide a quick venous drainage in between the venous occlusions. Subjects rested for 15 minutes before the measurements. The probe was attached on the lateral part (over the lateral gastrocnemius muscle) of the subject’s calf. A pneumatic cuff was wrapped around the subject’s thigh. Venous occlusion was achieved by inflating the pneumatic cuff with a high pressure line within 2 seconds to a pressure of 60 mm Hg. Venous occlusion was held for 1 minute, after which the pneumatic cuff pressure was quickly released. The pressure curve was recorded with a digital manometer (Cole-Parmer Instruments, Vernon Hills, IL). After acquisition of the baseline data for 2 minutes, five consecutive venous occlusions were carried out separated by a 2-minute rest period.

Data Analysis.—The absolute values for absorption, scattering, and DPF were calculated (29,31) in three center regions according to the sensor (Fig 1) and the frequency-domain equations. Quantitative values for relative changes in oxyhemoglobin and deoxyhemoglobin in all 22 locations were obtained by combining the attenuation changes with the DPF data (32).

From the time traces, the following parameters were derived:

1. Concentration changes in oxyhemoglobin, deoxyhemoglobin, and total hemoglobin concentration, where $\Delta$ total hemoglobin concentration equals the sum of $\Delta$ oxyhemoglobin and $\Delta$ deoxyhemoglobin concentration ($\Delta$ signifies a concentration change during venous occlusion). The $\Delta$ oxyhemoglobin, $\Delta$ deoxyhemoglobin, and $\Delta$ total hemoglobin concentration levels were calculated by subtracting the mean value obtained during the last 10 seconds before venous occlusion (baseline value) from the mean value obtained during the last 10 seconds of the venous occlusion.

2. Hemoglobin flow. We calculated the hemoglobin flow from the first 15 seconds of the increase in total hemoglobin concentration by using the slope of a linear regression, as follows: slope(total hemoglobin concentration)/10, in micromoles per 100 mL/min. It is calculated according to the plethysmography method (33). We give hemoglobin flow rather than blood flow (in milliliters of blood per 100 g/min) because it is the relevant parameter with respect to oxygenation because hemoglobin is the main oxygen carrier in blood and the oxygen content in the plasma is negligible. It is a strength of our method that hemoglobin flow can be determined. To compare our data with that in the literature, blood flow was calculated assuming a standard value for hemoglobin concentration in blood (14.1 g per 100 mL) because we did not take blood samples (34).

3. SvO$_2$, which was calculated with a linear regression of oxyhemoglobin and total hemoglobin concentration during the first 15 seconds of the venous occlusion, as follows: $\text{SvO}_2 = 100 \times \text{slope(oxyhemoglobin)} / \text{slope(total hemoglobin)}$, in percentage.

4. VO$_2$. Two methods were used to calculate the VO$_2$. First, we used the increase in deoxyhemoglobin concentration, which was calculated with linear regression over the entire period of the venous occlusion. Deoxyhemoglobin is mainly generated by the conversion of oxyhemoglobin into deoxyhemoglobin due to VO$_2$. The following formula is used to calculate VO$_2$: $\text{VO}_2 = 0.0645 \times 1.39/10.4 \times (\text{slope[deoxyhemoglobin]} - (100 - \text{SaO}_2)/100 \times \Delta[\text{total hemoglobin}])$, in milliliters per 100 g/min, where 0.0645 g/$\mu$mol is the weight of 1 $\mu$mol hemoglobin, 1.39 mL/g the volume of O$_2$ bound by 1 g of hemoglobin, and 10.4 [100 g/L] the specific weight of muscle tissue. The term “100 – SaO$_2$/100” represents the amount of deoxyhemoglobin in the arterial blood, which is small compared to the term “slope(deoxyhemoglobin).” A value of 97% was assumed for SaO$_2$ (35). Second, we calculated the VO$_2$ by using the following equation: $\text{VO}_2 = \text{hemoglobin flow} \times (\text{SaO}_2 - \text{SvO}_2)/100 \times 0.0645 \times 1.39/10.4$, in milliliters per 100 g/min.

All mentioned parameters were calculated for each of the 22 locations. Averages were taken for proximal and distal regions and medial and lateral regions. We also calculated the global mean values.

VO$_2$ can be determined with venous and arterial occlusion. The method used to calculate VO$_2$ from the conversion of oxyhemoglobin to deoxyhemoglobin during venous occlusion has been validated against an invasive technique (36), and both methods provide comparable results (37). We chose the venous occlusion method because it has the advantage that it is not painful, does not traumatize the tissue—neither directly through cuff pressure nor indirectly through tissue ischemia—and can be easily repeated. Moreover, it offers the unique feature of measuring hemoglobin flow and VO$_2$ simultaneously.

The test-retest variability (TRV) was calculated with analysis of variance for all five venous occlusions and for the last four venous occlu-
sions separately. To test for the statistical significance of the differences, we used the Wilcoxon test for paired data (SPSS 11.0, SPSS, Chicago, IL).

RESULTS

The global (mean over all measurements) values were as follows: Δoxyhemoglobin concentration, 6.23 μmol/L ± 3.55; Δdeoxygenhemoglobin concentration, 2.08 μmol/L ± 1.34; Δtotal hemoglobin concentration, 8.31 μmol/L ± 4.85; hemoglobin flow, 1.27 μmol per 100 mL/ min ± 0.88; blood flow, 0.56 mL per 100 g/min ± 0.38; VO₂, 0.016 mL per 100 g/min ± 0.011; SvO₂, 77.6% ± 5.9; and ATT, 10.0 mm ± 4.3.

In particular, when comparing proximal and distal regions all parameters showed highly significant differences (Table). We also found differences between medial and lateral regions. The regional mean values of Δoxyhemoglobin, Δdeoxygenhemoglobin, Δtotal hemoglobin concentrations, hemoglobin flow, VO₂, SvO₂ and ATT over all legs are given in the Table. The mean DPF was 5.55 ± 1.28 at 758 nm and 5.45 ± 1.16 at 830 nm.

From the data, we created maps for the different parameters by means of back projection. Maps of the mean values of Δoxyhemoglobin, Δdeoxygenhemoglobin, Δtotal hemoglobin concentrations, hemoglobin flow, VO₂ and SvO₂ for the left and right leg are shown in Figure 2. The Δoxyhemoglobin, Δtotal hemoglobin concentrations, and hemoglobin flow for the left leg were most pronounced in the proximal-medial part of the calf. In the right leg, the changes were more distributed over the mapped region. The highest magnitude, however, was in the proximal region, and the lowest values were in the distal-lateral region. The changes in the Δdeoxygenhemoglobin concentration and VO₂ appeared similar for both legs and were much lower in the distal compared to the proximal region. The highest SvO₂ values for the left leg were in the distal and proximal-medial regions. A similar pattern was seen in the right leg, but the pattern was not as clear.

TRV

TRV for all parameters was determined for all five and the last four venous occlusions because we generally observed significantly higher values for the second to fifth venous oc-
clusion compared to the first venous occlusion. The TRV over all five venous occlusions was between 13% and 17% for all parameters except hemoglobin flow. The TRV over the last four venous occlusions was between 11% and 15%. The TRV for hemoglobin flow was 32% and 28%, respectively.

DISCUSSION

We generated maps of hemodynamics and oxygenation—that is, Δoxyhemoglobin, Δdeoxygenated hemoglobin, Δtotal hemoglobin concentrations, hemoglobin flow, VO₂, and SvO₂—in the human calf muscle at rest. For all measured parameters, we observed a spatial heterogeneity not only between subjects but also within individual legs. In the following sections, we discuss possible origins for the heterogeneities.

The penetration depth of near-infrared light is limited and the measured values reflect the parameters of all traversed layers of tissue. Thus, the ATT influences the measurement (16,38–41). We found that the spatial heterogeneity for an ATT of more than 10 mm is smaller than that for an ATT of less than 10 mm. This indicates that the spatial heterogeneity has its origin in the muscle and not in the adipose tissue. The spatial distribution may reflect a different anatomy (ie, capillary density) or a different hemodynamic response to the venous occlusion (ie, redistribution of blood).

Maps

Our results demonstrate for each determined parameter a spatially heterogeneous distribution over the measured region. A spatial heterogeneity was previously described for mean tissue oxygen saturation in the vastus lateralis (24) and blood flow in the quadriceps muscle (25). In addition, herein we report the spatial distribution of hemoglobin flow, VO₂, and SvO₂.

The maps for hemoglobin flow, Δtotal hemoglobin concentration, and Δoxyhemoglobin concentration show similar patterns. During venous occlusion, the venous outflow is blocked while the arterial inflow is sustained and, therefore, total hemoglobin concentration increases. Because the arterial blood circulating to the tissue is mostly oxygenated and only a minor part of the oxygen is consumed by the muscle tissue at rest, the major hemoglobin fraction constituting the increase in total hemoglobin concentration level is oxyhemoglobin.

The spatial distribution of VO₂ and Δdeoxygenated hemoglobin concentration appears similar. This can be understood because deoxyhemoglobin is mostly generated by VO₂, that is, the turnover of oxyhemoglobin to deoxyhemoglobin during venous occlusion. In fact, the VO₂ can be calculated from the increase in deoxyhemoglobin concentration as stated earlier.

The maps also demonstrate the heterogeneity in the correlation of hemoglobin flow and VO₂ in the tissue. First of all, the spatial distribution of hemoglobin flow and VO₂ is not congruent. That in areas with high VO₂, hemoglobin flow is also high turns out not to be generally valid. Moreover, hemoglobin flow can vary in regions with similar VO₂. This shows that hemoglobin flow is not regulated by oxygen demand only.

Our results show that VO₂ and hemoglobin flow are not equally distributed. This is in accordance with the findings of Casavola et al (42), who found that VO₂ is not necessarily correlated with blood flow. Beekvelt et al (8), who obtained measurements in a single location, observed that blood flow did not increase by the same factor as VO₂.

In the resting muscle, one could think of a homogeneously distributed VO₂. Our maps clearly show that VO₂ varies depending on the location. One of the underlying reasons for a spatially distributed VO₂ may be found in the proportion of the different muscle fiber types within the probed tissue volume. It is well known from the literature that, during exercise, the oxygen demand differs for different types of muscle fibers (43); however, no data were found for the resting muscle.

SvO₂ reflects the relationship between hemoglobin flow and VO₂. The SvO₂ is high in regions with a low VO₂ and high hemoglobin flow and those with a low VO₂ and low hemoglobin flow. In regions with a high VO₂, however, SvO₂ decreases with decreasing hemoglobin flow.

The reason why the maps for the left and right legs are not symmetric cannot be clarified yet. It may be influenced by the fact that we always measured the right leg first while throughout the entire measurement both legs were elevated, or it may be due to physiologic variations.

When VO₂ was calculated by using the hemoglobin flow and arteriovenous oxygen saturation difference, it was found to be in close quantitative agreement (mean deviation, 6.8%) with the VO₂ values presented earlier calculated from the increase in deoxyhemoglobin concentration. This also implies that our data are internally consistent. In the literature, we found a wide range of values for VO₂ in the human calf muscle: 0.026–0.14 (42), 0.050–0.097 (44), 0.01 (45), 0.13 (10), and 0.047 mL per 100 g/min (12). In a PET study (46), those values ranged from 0.08 to 0.66 mL per 100 g/min. Our value of 0.016 mL per 100 g/min is in the low range of these values. For blood flow, we also found a wide range of values in the literature: 0.28–0.57 (42), 2.3 (47), 0.70 (45), 1.45 (10), and 0.65 mL per 100 g/min (12). In a PET study (46), those values ranged from 2.2 to 9.5 mL per 100 g/min. Our mean value was 0.56 mL per 100 g/min, which is again in the lower range of those given in the literature. A likely reason for the large range of values found in the literature is the spatial dependence. Our values are in the lower range because our measured mean DPF was 5.5 compared to a lower value of 4 from in the literature. If we considered other methodologic aspects such as location and time period of the linear regression (we chose a long period of 60 seconds to obtain stable estimates), we can account for a factor of ~2.6 by which our values are lower compared to the ones in the literature.

Precision and TRV

The measurement of VO₂ with venous occlusion has been validated against an invasive technique (36) or phosphorus 31 MR spectroscopy (48). The measurements of SvO₂ (49) and blood flow (35,50) have also been validated against other methods.

The precision or TRV for all parameters was tested over all five and over the last four venous occlusions because the values for all parameters except VO₂ and SvO₂ were lower in the first compared to values of the follow-
ing four venous occlusions. We observed an increase in the magnitude of $\Delta$oxyhemoglobin, $\Delta$deoxygenyhemoglobin, $\Delta$total hemoglobin concentrations, and hemoglobin flow values both from the first to the second venous occlusion, which was significant, and from the second to the third, which was only significant for $\Delta$total hemoglobin concentration. The increasing magnitude in concentration changes over repetitive venous occlusions was also observed by other authors but not discussed (35,51). During the first venous occlusion, blood vessels dilate and capillaries become recruited, which increases the blood volume. Within a 2-minute recovery time between the venous occlusions, vessels may still be predistended and recruited so that with the following venous occlusion, blood can be more easily and quickly accumulated in the capillary bed and smaller vessels. The smaller increase from the second to third venous occlusion may occur because either the vessels do not dilate further or the increase in the intravascular pressure does not exceed the threshold to recruit more capillaries.

The TRV over all five venous occlusions was between 13% and 17% for all parameters except hemoglobin flow (32%). The TRV over the last four venous occlusions was 11%–15% for all parameters except hemoglobin flow (28%). The TRV over the four last venous occlusions is lower for the physiologic reason mentioned earlier and reflects the methodologic error of the measurement more clearly. For measurements under physiologic conditions, a TRV of up to 20% is considered to be good. In the literature, a TRV of 22.3% was found for VO$_2$ in the foot (44), and a TRV of 10.5% was found for hemoglobin flow in the forearm (35). Because these values were obtained from different tissues than in the current study, they cannot be compared directly; however, our values are within the same range.

The TRV is only a true measure of the error of measurement if the measured variable is always completely constant. In our case, it overestimates the error because there are always genuine physiologic changes (eg, slow vasomotion and variation in systemic blood pressure).

The precision of measurements of physiologic parameters can be improved by increasing the number of repetitions. In the case of noninvasive ness of the applied method, this requirement can easily be fulfilled.

**Myoglobin**

NIRS cannot differentiate between hemoglobin and myoglobin due to the similar absorption spectra, that is, oxyhemoglobin and oxymyoglobin and deoxyhemoglobin and deoxymyoglobin, respectively, are confounded. The contribution of myoglobin to the signal, however, is 5%–10%, an amount that can be considered negligible (39). Moreover, myoglobin is not relevant in the calculation of hemoglobin flow or blood flow because its concentration can be assumed to remain constant during the venous occlusion. Thus, a change in total hemoglobin concentration can be attributed solely to the hemoglobin fractions. In terms of VO$_2$, it does not matter either for two reasons. First, we measured at rest, under the condition of sufficient oxygen supply, and a desaturation of myoglobin is not likely to occur. Second, even in the case of a myoglobin desaturation this factor would not falsify the results because the term VO$_2$ covers oxygen consumption whether it results from a desaturation of hemoglobin or myoglobin. The only parameters that could be affected are the oxyhemoglobin/deoxyhemoglobin concentration ratio (ie, $\Delta$oxyhemoglobin concentration, $\Delta$deoxygenyhemoglobin concentration and SvO$_2$).

**DPF**

An established method is to take DPF values from the literature although some authors have pointed out the shortcoming of this method (51). From our data, it can be seen that the DPF varies depending on the ATT. We also observed that the DPF decreased during the venous occlusion by 2%–5%, which was described previously (27). We found a considerable variation in DPF, particularly between subjects. These findings demonstrate the importance of measuring the DPF continually by using the frequency-domain method to obtain accurate quantitative values.

**Limitations, Potential Clinical Applications, and Outlook**

The main limitations of determining the hemodynamics and oxygenation in muscle by using NIRS combined with venous occlusion are the intermittent nature and the relatively low spatial resolution. The measurement is intermittent and yields one value per venous occlusion. However, the values are likely to be reproducible even if measured on different days (44,50). The spatial resolution is on the order of 1 cm and is two-dimensional. The values, however, are representative of an average of the sampled volume.

There are many potential clinical applications of NIRS, particularly when addressing abnormalities in the blood circulation and oxygenation. The set-up is appreciated by patients and personnel alike due to its advantageous features and it provides useful, clinically relevant information. Examples of applications are the localization of ischemia in tissue, the intraoperative assessment of reperfusion at the capillary level, and the early detection of peripheral vascular disease to motivate preventative measures.

Work is currently under way to overcome the above limitations and, in the future, NIRS will provide continuous and three-dimensional measurements of hemoglobin flow, SvO$_2$, total hemoglobin concentration, and VO$_2$.

In conclusion, the distribution of hemoglobin flow, VO$_2$, SvO$_2$, and concentration changes in oxyhemoglobin, deoxyhemoglobin, and total hemoglobin during venous occlusion differed significantly depending on the location in the calf muscle. If this distribution is of interest, mapping, instead of single location measurements, with NIRS is recommended. ATT had an influence on the measurements and, therefore, must be measured. Because NIRS offers the unique capability to measure and monitor hemodynamics and oxygenation simultaneously in small blood vessels (eg, arterioles, capillaries, and venules), several centimeters deep in the tissue, it is a valuable diagnostic tool and can be combined with other methods.

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