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Localized irregularities in hemoglobin flow and oxygenation in calf muscle in patients with peripheral vascular disease detected with near-infrared spectrophotometry

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Purpose: **Near-infrared spectrophotometry is used to measure flow, concentration, and oxygenation of hemoglobin in arterioles, capillaries, and venules several centimeters deep in tissue. The purpose of this study was to investigate the distribution of flow, concentration, and oxygenation of hemoglobin in calf muscle in patients with documented peripheral arterial occlusive disease (PVD), patients with risk factors for PVD ,and healthy younger subjects at rest.**
Method: **With a frequency-domain near-infrared spectrophotometer and a specially designed probe, we generated maps at 22 locations simultaneously of hemoglobin flow, concentration, and oxygenation, with the venous occlusion method. Eight legs of 7 patients with diagnosed PVD (PVD group), 10 legs of 8 patients with normal ankle-brachial index but with risk factors for PVD (RF group), and 16 legs of 8 healthy subjects (H group) were studied.**
Results: **Global mean values were significantly ($P < .05$) different between the three groups for oxygen consumption (PVD group, 0.027 ± 0.009 mL/100 g/min; RF group, 0.038 ± 0.017 mL/100 g/min; H group, 0.022 ± 0.020 mL/100 g/min), venous oxygen saturation (PVD, 59.7% ± 15.4%; RF, 69.6% ± 10.5%; H, 80.8% ± 4.5%), and, at 60 s of venous occlusion, concentration changes in oxyhemoglobin (PVD, 4.48 ± 3.25 μmol/L; RF, 8.44 ± 2.33 μmol/L; H, 6.85 ± 4.57 μmol/L), deoxyhemoglobin (PVD, 3.60 ± 0.73 μmol/L; RF, 4.39 ± 1.30 μmol/L; H, 2.36 ± 1.79 μmol/L), and total hemoglobin (PVD, 8.07 ± 3.83 μmol/L; RF, 12.83 ± 2.75 μmol/L; H, 9.21 ± 6.34 μmol/L). No significant difference was found between the three groups for hemoglobin flow (PVD, 0.92 ± 0.69 μmol/100 mL/min; RF, 1.68 ± 0.50 μmol/100 mL/min; H, 1.44 ± 1.17 μmol/100 mL/min) and blood flow (PVD, 0.45 ± 0.28 mL/100 g/min; RF, 0.77 ± 0.21 mL/100 g/min; H, 0.62 ± 0.50 mL/100 g/min). All parameters featured a distribution dependent on location.**
Conclusion: **Mean value for venous oxygen saturation was higher in healthy subjects compared to patients with documented PVD. In patients with PVD, areas of lower oxygenation were clearly discernible. At distal locations of calf muscle, significant correlations between reduced hemoglobin flow, venous oxygen saturation, oxyhemoglobin, and total hemoglobin and reduced ankle-brachial index were found. Maps revealed localized irregularities in oxyhemoglobin, total hemoglobin, and venous oxygen saturation in patients with PVD.**
Near-infrared spectrophotometry is a noninvasive bedside technique that can enable determination of blood flow and oxygenation in tissue and may provide a method for evaluating patients with PVD. (J Vasc Surg 2003;37:1017-26.)

In recent years, near-infrared spectrophotometry (NIRS) has been used increasingly to measure flow, concentration, and oxygenation of hemoglobin in the brain,[1,2] skeletal muscle, muscle flaps,[3,4] and individual organs.[5] Measurements of skeletal muscle have been performed in athletes[6] and in patients with myopathies,[7-9] heart failure,[10-12] and peripheral arterial occlusive disease (PVD).[13-19] NIRS has also been used to monitor blood perfusion and oxygenation during surgery[20] and in intensive care medicine.[21,22]

NIRS is based on the principle of light attenuation by tissue, and it has the unique ability to noninvasively measure tissue oxygenation and hemoglobin concentration at a depth of several centimeters, specifically, at the level of arterioles, capillaries, and venules.[23] Real-time measurements (6 Hz), good spatial resolution (5 mm), bedside feasibility, and high reproducibility could make this method a valuable tool to provide insight into the distribution of hemoglobin flow, concentration, and oxygenation in the clinical setting.

Most NIRS instruments measure only at one discrete location. However, previous studies indicated that hemoglobin flow, concentration, and oxygenation in muscle

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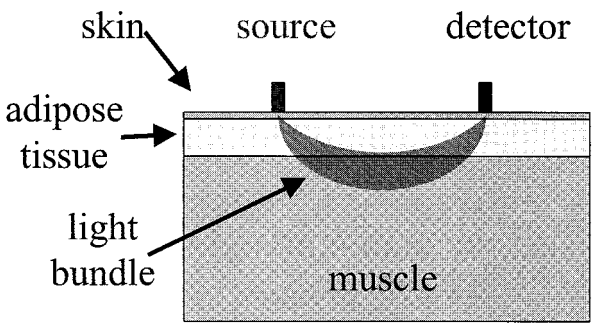

Fig 1. Light is emitted at source location. It penetrates tissue before being detected at a given distance from source. *Shaded area* (light bundle) indicates approximate path of light.

demonstrate variability throughout the muscle at rest[24-26] and during exercise.[27]

The purpose of this study was to advance NIRS to generate maps and to demonstrate the spatial distribution of hemoglobin flow, concentration, and oxygenation in human calf muscle in patients with PVD, patients with risk factors for PVD, and healthy subjects.

MATERIAL AND METHODS

Principle

NIRS is based on the principle that near-infrared light propagates through biologic tissue and can be re-collected by detectors at a given distance from the light source (Fig 1). When NIR light is sent through tissue, only part of the emitted light reaches the detector, because tissue attenuates the propagating light; ie, it is absorbed or scattered. From the intensity of the re-collected light, information about the traversed tissue can be obtained and oxyhemoglobin (O_2Hb) and deoxyhemoglobin (HHb) concentrations can be determined with spectral analysis.

Instrument

Measurements were obtained with a frequency-domain spectrophotometer (Oxy-Imager, ISS, Champaign, Ill), which uses intensity-modulated light at a frequency of 110 MHz.[28] In brief, the instrument uses laser diodes at two wavelengths, 830 and 758 nm, corresponding to the high absorption of O_2Hb and HHb, respectively. The light of 32 laser diodes (16 per wavelength) is conducted from the instrument to the tissue and back with optical glass fibers.

Sensor

An area 18.5 × 6 cm (Fig 2) is covered by the sensor and enables simultaneous measurements at 22 locations.

Protocol

To measure flow, concentration, and oxygenation of hemoglobin, we used the venous occlusion method, which, in comparison with ischemia tests, enables us to measure venous oxygen saturation (Svo_2), hemoglobin flow, and oxygen consumption (Vo_2) simultaneously. Moreover, it is not painful and can be easily repeated. Calculation of Vo_2 with venous occlusion has been validated against an invasive technique,[29] with both the venous and arterial occlusion methods providing similar results.[30] Measurements of Svo_2[31] and blood flow[32] have been validated against other methods.

For 15 minutes before measurements were obtained, all patients and healthy subjects rested in a quiet room with constant temperature. They reclined comfortably in a supine position with both legs elevated slightly above heart level to provide quick venous drainage between venous occlusions. The sensor was placed and secured with an elastic band along the lateral side of the calf (lateral gastrocnemius muscle), and a pneumatic cuff was wrapped around the thigh. Venous occlusion was induced (within 2 seconds) by inflating the pneumatic cuff to a pressure of 60 mm Hg. Venous occlusion was maintained for 60 seconds for the first venous occlusion and for 180 seconds for the final venous occlusion. Between 3 and 5 venous occlusions were performed. At the end of each venous occlusion the pneumatic cuff pressure was quickly released. The pressure curve was recorded with a digital manometer (Cole Parmer, Vernon Hills, Ill). Consecutive venous occlusions were separated by 2-minute rest periods. This protocol was approved by the Institutional Review Board of the University of Illinois at Urbana-Champaign (IRB #94125) and the Veterans Affairs North Texas Health Care System, Dallas, Tex.

Measurement of adipose tissue thickness

Adipose tissue thickness of the calf was measured with a skinfold caliper (Lange, Beta Technology, Inc, Santa Cruz, Calif).

Measurement of ankle-brachial index

According to standard procedures of the Veterans Affairs North Texas Health Care System, ankle-brachial index (ABI) was determined by the ratio of systolic blood pressure at the ankle (dorsalis pedis artery or posterior tibial artery) over systolic blood pressure at the arm (brachial artery).

Data analysis

Relative changes in [O_2Hb] and [HHb] in all 22 locations were calculated from light attenuation changes with frequency-domain NIRS algorithms.[33-35]

Two different time courses of venous occlusion are presented in Fig 3, and the following parameters were derived from time tracings of changes in [O_2Hb] and [HHb]:

1. Hemoglobin concentration changes in oxyhemoglobin ($\Delta[O_2Hb]$), deoxyhemoglobin (Δ[HHb]), and total hemoglobin (Δ[tHb]), where Δ[tHb] equals the sum of $\Delta[O_2Hb]$ and Δ[HHb]. Increases in $\Delta[O_2Hb]$, Δ[HHb], and Δ[tHb] were calculated by subtracting the mean baseline value obtained from the last 10 seconds before the venous occlusion from the mean value

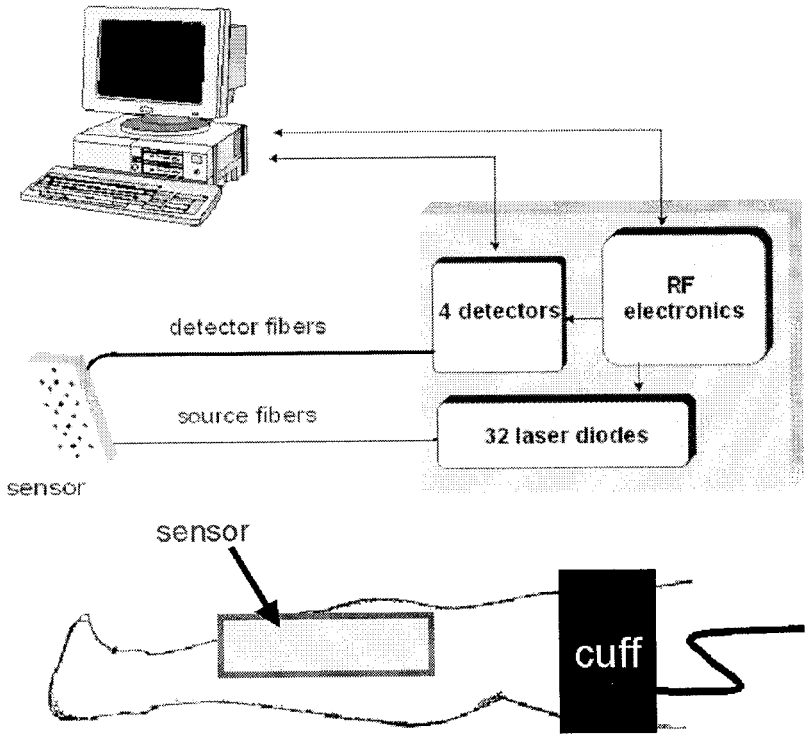

Fig 2. System setup. Sensor is placed on lateral calf, where it interrogates an area 18.5 × 6 cm.

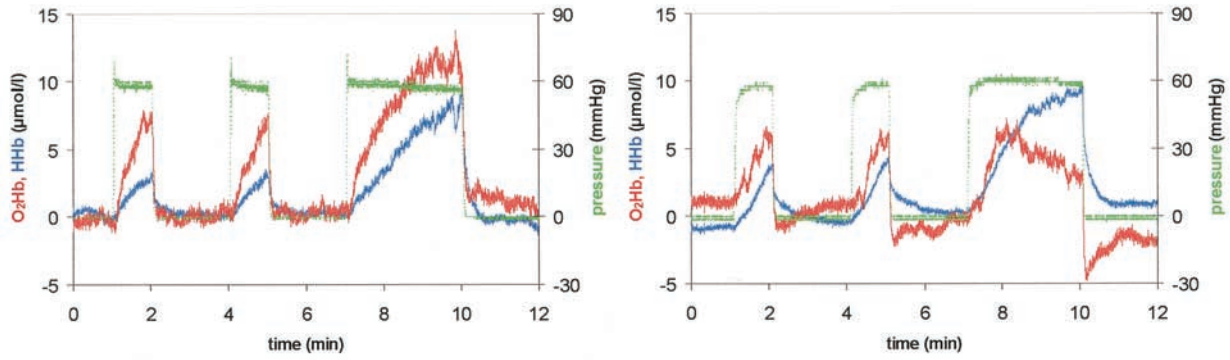

Fig 3. Two tracings of changes in [O_2Hb] and [HHb] and pneumatic cuff pressure during venous occlusion. The two diagrams display tracings obtained from two different subjects at the same location on calf muscle. In all venous occlusions [O_2Hb] and [HHb] increase *(left)*, while in one venous occlusion of 180-second duration *(right)* [O_2Hb], after initial increase, decreases.

between 50 and 60 seconds and between 170 and 180 seconds, respectively, of the venous occlusion.

2. Hemoglobin flow was calculated from the first 15 seconds of the increase in [tHb] with the slope of a linear regression.[32] Blood flow was calculated with the hemoglobin blood concentration.
3. Svo_2 was calculated with a linear regression of [O_2Hb] and [tHb] during the first 15 seconds of venous occlusion.[31]
4. Vo_2 was calculated with linear regression for the increase in [HHb] over the first 15 seconds of venous occlusion,[36] because increase in [HHb] mainly reflects conversion of O_2Hb into HHb generated by Vo_2.

Statistics

Correlation to ankle-brachial index. Since at high ankle pressure the ABI may be falsely too high,[37] ABI

Table I. Hemodynamic and oxygenation parameters

	Patients with documented PVD	*Patients at risk for PVD*	*Healthy subjects*	P	*A*	*B*	*C*
Number of legs	8	10	16				
$\Delta[O_2Hb]$ 60 s (μmol/L)	4.48 ± 3.25	8.44 ± 2.33	6.85 ± 4.57	.038	*	NS	NS
Δ[HHb] 60 s (μmol/L)	3.60 ± 0.73	4.39 ± 1.30	2.36 ± 1.79	.001	NS	†	†
Δ[tHb] 60 s (μmol/L)	8.07 ± 3.83	12.83 ± 2.75	9.21 ± 6.34	.022	*	NS	*
$\Delta[O_2Hb]$ 180 s (μmol/L)	8.06 ± 6.11	9.21 ± 4.28	9.80 ± 4.55	NS			
Δ[HHb] 180 s (μmol/L)	10.37 ± 2.76	9.48 ± 2.95	6.38 ± 3.07	.002	NS	†	*
Δ[tHb] 180 s (μmol/L)	18.42 ± 7.95	18.69 ± 5.02	16.18 ± 7.32	NS			
HF (μmol/100 mL/min)	0.92 ± 0.69	1.68 ± 0.50	1.44 ± 1.17	NS			
BF (mL/100 g/min)	0.45 ± 0.28	0.77 ± 0.21	0.62 ± 0.50	NS			
V_{O_2} (mL/100 g/min)	0.027 ± 0.009	0.038 ± 0.017	0.022 ± 0.020	.009	NS	NS	*
S_{VO_2} (%)	59.7 ± 15.4	69.6 ± 10.5	80.8 ± 4.5	.00009	NS	‡	‡

Value represent mean ± SD.

Δ, Increase during venous occlusion after 60 or 180 seconds; *O_2Hb*, oxyhemoglobin; *HHb*, deoxyhemoglobin; *tHb*, total hemoglobin; *HF*, hemoglobin flow; *BF*, blood flow; *VO_2*, oxygen consumption; S_{VO_2}, venous oxygen saturation. *P*, Significance between all three groups; *A*, significance of difference between patients with documented PVD and patients with risk factors for PVD; *B*, significance of difference between patients with documented PVD and healthy subjects; *C*, significance of difference between patients with risk factors for PVD and healthy subjects; *NS*, not significant.

Statistical comparisons between two groups were only calculated when Kruskal-Wallis test showed significant variability (*P*) between the three groups.

*$P < .05$.

†$P < .005$.

‡$P < .0005$.

values in patients with ankle pressure more than 175 mm Hg were excluded. Both groups of patients were combined to get a broader range of ABI values. The significance of the correlation was determined with Spearman's rho.

Differences. To test for statistical significance of differences, we used the Kruskal-Wallis test for data with more than two groups, the Wilcoxon test for paired data, and the Mann-Whitney *U* test for unpaired data.

Subjects

Patients with PVD. Legs with diagnosed PVD were identified with either ABI less than 0.9, status after surgery, or pathologic findings at angiography. Eight legs of 7 male patients with PVD with mean age of 69 years (range, 50-77 years) were included in the study.

Patients at risk. The study included 10 legs of 8 male patients with mean age of 66 years (range, 51-79 years) with normal ABI (>0.9) but one or more risk factors for PVD: cardiovascular risk factors including hypertension, hyperlipidemia, tobacco smoking, diabetes mellitus, or coronary heart disease.

Healthy subjects. Sixteen legs of 8 healthy subjects (5 male, 3 female) with a mean age of 35 years (range, 23-44 years) were measured.

All subjects and patients gave written informed consent prior to measurement.

RESULTS

Mean values per leg. Global mean values for flow, concentration, and oxygenation of hemoglobin parameters for all three groups of subjects are shown in Table I. There were significant differences between the three groups, particularly for V_{O_2} and S_{VO_2}, whereas hemoglobin flow and blood flow were not significantly different.

We found a decrease in $\Delta[O_2Hb]$ during the later part of the 180 seconds of venous occlusion in 39.8% of measured locations in patients with PVD and in 66.8% of patients with risk factors for PVD, which is much greater than in healthy subjects (21.9%).

From the repeated measurements we calculated an error of measurement of 7% to 10% for $\Delta[O_2Hb]$, Δ[HHb], and Δ[tHb] and 17% to 20% for hemoglobin flow, blood flow, V_{O_2}, and S_{VO_2} in patients with PVD and patients with risk factors for PVD. In healthy subjects the error of measurement was similar.

In healthy subjects the difference between the left and right legs was not significant for any parameter.

Correlation with ankle-brachial index. ABI correlated with $\Delta[O_2Hb]$ at 60 seconds of venous occlusion ($P = .010$), Δ[tHb] at 60 seconds of venous occlusion ($P = .008$), S_{VO_2} ($P = .019$), and hemoglobin flow ($P = .003$) measured in the distal region. The correlation between ABI and Δ[tHb], hemoglobin flow, and S_{VO_2} is shown in Fig 4. The correlation for $\Delta[O_2Hb]$ after 60 seconds of venous occlusion is similar to that for Δ[tHb] after 60 seconds of venous occlusion and is therefore not depicted. Inasmuch as in arteriosclerosis the ABI can be falsely high, data for patients with ABI higher than 1.1 and ankle pressure higher than 175 mm Hg are not shown in these graphs.

Maps. There were significant differences between the proximal and distal regions of calf muscle in healthy subjects (Table II). These differences were much less pronounced in patients.

Maps, corresponding to the area of the sensor, for 1 healthy subject and 5 patients are shown in Figs 5 and 6. Localized irregularities in hemoglobin concentration and oxygenation parameters are clearly visible in the maps for the patients.

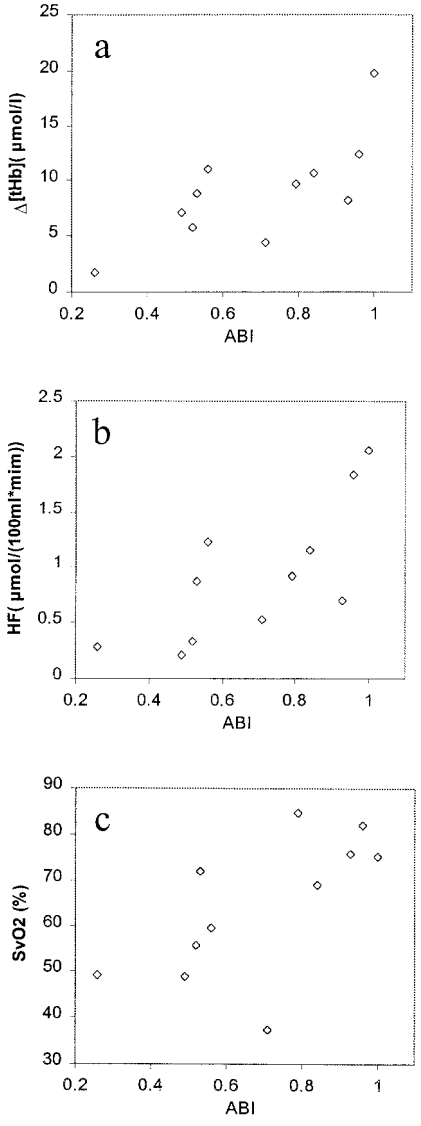

Fig 4. Correlation between ankle-brachial index *(ABI)* and Δ[tHb], hemoglobin flow *(HF)*, and Svo_2 for distal region of calf muscle.

Variability for Δ[HHb] after 60 and 180 seconds of venous occlusion, Δ[tHb] after 180 seconds of venous occlusion, Vo_2, and Svo_2 within maps was significantly higher for patients compared with healthy subjects.

DISCUSSION

Hemoglobin flow, blood flow, and tHb accumulation. Although at rest mean blood flow in patients with documented PVD (0.45 ± 0.28 mL/100 g/min) was considerably lower than in patients with risk factors for PVD (0.77 ± 0.21 mL/100 g/min) or healthy subjects (0.62 ± 0.50 mL/100 g/min), this difference was not statistically significant. This finding and the values measured for blood flow are in agreement with results from Kooijman et al,[17] who reported median blood flow of 0.56 mL/100 g/min in patients and 0.65 mL/100 g/min in healthy subjects. Another study[38] found mean blood flow of 1.34 mL/100 g/min in patients and 0.70 mL/100 g/min in healthy subjects. In a study that used positron-emission tomography (PET),[39] mean blood flow was 2.3 to 8.4 mL/100 g/min in patients and 2.2 to 9.3 mL/100 g/min in control subjects. The results given in the literature were generated by single location measurement, and no correlation with stage of PVD was established.

With respect to oxygenation, hemoglobin flow is more relevant than blood flow, which comprises hemoglobin and plasma, because hemoglobin is the main oxygen carrier in the blood and the oxygen content in the plasma is negligible. A strength of our method is that it determines hemoglobin flow.

We found a correlation with ABI when distal hemoglobin flow values were considered. With decreasing ABI, hemoglobin flow decreased (Fig 4, *B*). This result indicates that with progressing PVD, which is reflected by decreasing ABI, perfusion decreases. As a consequence of diminished perfusion, the decreasing Svo_2 with declining ABI can be understood (Fig 4, *C*).

Mean Δ[tHb] in 60 seconds, which is related to hemoglobin flow and blood flow because it signifies rate of accumulation of hemoglobin in tissue, shows a significant difference between patients with risk factors for PVD and healthy subjects or patients with PVD. When the distal region was considered, with increasing ABI a larger increase in Δ[tHb] after 60 seconds was observed (Fig 4, *A*), which can be explained by the impaired hemoglobin flow in patients with low ABI.

VO_2 and HHb accumulation. Mean Vo_2 was higher in patients with risk factors for PVD than in the healthy subjects, 0.038 ± 0.017 mL/100 g/min and 0.022 ± 0.020 mL/100 g/min, respectively. In patients with documented PVD, Vo_2 was 0.027 ± 0.009 mL/100 g/min), similar to that in healthy subjects. Krageli et al[40] found that Vo_2 was not significantly lower in patients with PVD (0.065 ± 0.019 mL/100 g/min) compared with control subjects (0.071 ± 0.019 mL/100 g/min). Kooijman et al[17] reported a higher median value for Vo_2 in patients with PVD (0.055 mL/100 g/min) compared with control subjects (0.047 mL/100 g/min), although the difference was not significant at rest. After walking exercise, patients had higher Vo_2 compared with control subjects (7.60 mL/100 g/min vs 3.30 mL/100 g/min). The 75th percentile was considerably higher in patients compared with control subjects. This indicates that differences between the two groups may be larger, had a mean been calculated. Other studies reported mean Vo_2 values of 0.16 mL/100 g/min for patients and 0.10 mL/100 g/min for healthy subjects,[38] median Vo_2 of 0.1 mL/100 g/min for patients (interquartile range, 0.04-0.16 mL/100 g/min) and 0.2 mL/100 g/min (interquartile range, 0.16-0.24 mL/100 g/min) for healthy subjects, and a broad overlap between the two groups,[19,38] and a Vo_2 range of 0.08 to 0.15

Table II. Proximal-distal differences for parameters in patients with documented PVD and with risk factors for PVD and in healthy subjects

	Patients with documented PVD	*Patients at risk for PVD*	*Healthy subjects*
Δ[O_2Hb] 60s (μmol/L)	0.86 ± 3.59	2.66 ± 7.87	3.38 ± 3.22†
Δ[HHb] 60s (μmol/L)	1.14 ± 1.56	0.94 ± 2.80	1.18 ± 0.67†
Δ[tHb] 60s (μmol/L)	2.01 ± 3.75	3.60 ± 9.38	4.56 ± 3.36†
Δ[O_2Hb] 180 s (μmol/L)	2.54 ± 3.08*	−0.13 ± 6.23	3.88 ± 4.46*
Δ[HHb] 180 s (μmol/L)	3.03 ± 6.07	0.03 ± 7.78	2.19 ± 2.12†
Δ[tHb] 180 s (μmol/L)	5.57 ± 6.78	−0.09 ± 9.87	6.07 ± 3.63†
HF (μmol/100 mL/min)	0.52 ± 1.04	0.37 ± 1.01	0.70 ± 0.60†
BF (mL/100 g/min)	0.23 ± 0.45	0.15 ± 0.46	0.30 ± 0.26†
Vo_2 (mL/100 g/min)	0.003 ± 0.021	0.013 ± 0.024	0.011 ± 0.009†
Svo_2 (%)	3.6 ± 17.8	−6.1 ± 7.7*	−3.9 ± 4.6†

Δ, increase during venous occlusion after 60 or 180 seconds; *O_2Hb*, oxyhemoglobin; *HHb*, deoxyhemoglobin; *tHb*, total hemoglobin; *HF*, hemoglobin flow; *BF*, blood flow; *VO_2*, oxygen consumption; Svo_2, venous oxygen saturation.
*$P < .05$.
†$P < .005$.

mL/100 g/min in patients and 0.08 to 0.66 mL/100 g/min in control subjects with PET measurement.[39] There is broad variation between studies. Our values are in the lower range because we averaged over a whole region, including regions with low Vo_2, ie, both the distal and lateral part of the calf muscle. Yet the origin of the higher Vo_2 in the patients remains unclear.

The mean Δ[HHb] at 60 seconds (Table I) was higher in the patients, which is related to increased Vo_2 in this group.

O_2Hb accumulation. To calculate blood flow, venous occlusion is usually maintained for no more than 60 seconds. When we extended occlusion time to 180 seconds after an initial increase, a decrease in [O_2Hb] appeared in some subjects and some locations, while [tHb] was still increasing. The declining [O_2Hb] indicates that arterial inflow cannot counterbalance conversion of O_2Hb into HHb because of Vo_2. The effect was observed three times more frequently in patients with risk factors for PVD than in healthy subjects. If it were facilitated by an insufficiency of oxygen supply, the higher Vo2 in patients with risk factors for PVD would enhance that effect.

Svo_2. Mean Svo_2 was highly significantly lower in patients with documented PVD or with risk factors for PVD compared with healthy subjects. It is noteworthy that the standard deviation was much wider in the two patient groups (Table I), which reflects the variety of disease stage in the patients. Lower tissue oxygen saturation in patients compared with a control group measured with NIRS was previously reported.[41]

Proximal-distal differences. In healthy subjects we found significantly higher values in the proximal region compared with the distal region of calf muscle for hemoglobin flow, blood flow (in agreement with the literature[42]), hemoglobin concentration increase, and Vo_2 (Table II). In patients the regional differences showed much more variability and were thus rarely significant (Table II). Regional differences can be explained by decreased muscle mass in the distal part.

Svo_2, in contrast, was significantly higher distally. Of interest, in patients with documented PVD, Svo_2 was lower distally, which indicates that oxygenation is lower distally. This correlates with the course of the disease.

Maps of hemoglobin accumulation and Svo_2. To illustrate the spatial distribution of the most important parameters in flow, concentration, and oxygenation of hemoglobin, we created maps by backprojection. To the best of our knowledge, the present study is the first to map hemoglobin flow, concentration, and oxygenation over a large area in calf muscle in patients with PVD.

The maps clearly show a spatial distribution of hemoglobin flow, concentration, and oxygenation. In a healthy subject (Fig 5), spatial heterogeneity in Δ[O_2Hb] and Δ[tHb] was noted, with the highest values proximal and medial. Svo_2 varies slightly depending on location, but remains within the physiologic range.

Comparison between subjects B and C (Fig 5) demonstrates that although the ABI is almost identical, the hemoglobin concentration changes and Svo_2 differ. In both subjects the patterns of the hemoglobin concentration changes and Svo_2 appear more heterogeneous than in the healthy subject. Svo_2 dips below the physiologic range in some locations. Of interest, these regions are not in the distal part of the calf muscle.

The maps for patients D, E, and F (Fig 6) express a different pattern of hemoglobin concentration change during venous occlusion. After an initial increase during venous occlusion, [O_2Hb] decreases. [tHb] increases continuously, indicating that arterial inflow is not blocked by cuff pressure. Nevertheless, the decrease in [O_2Hb] demonstrates that oxygen supply does not fill oxygen demand. Since Vo_2 did not change during venous occlusion, the most probable reason for the decrease in [O_2Hb] is insufficient perfusion pressure, which cannot counterbalance the

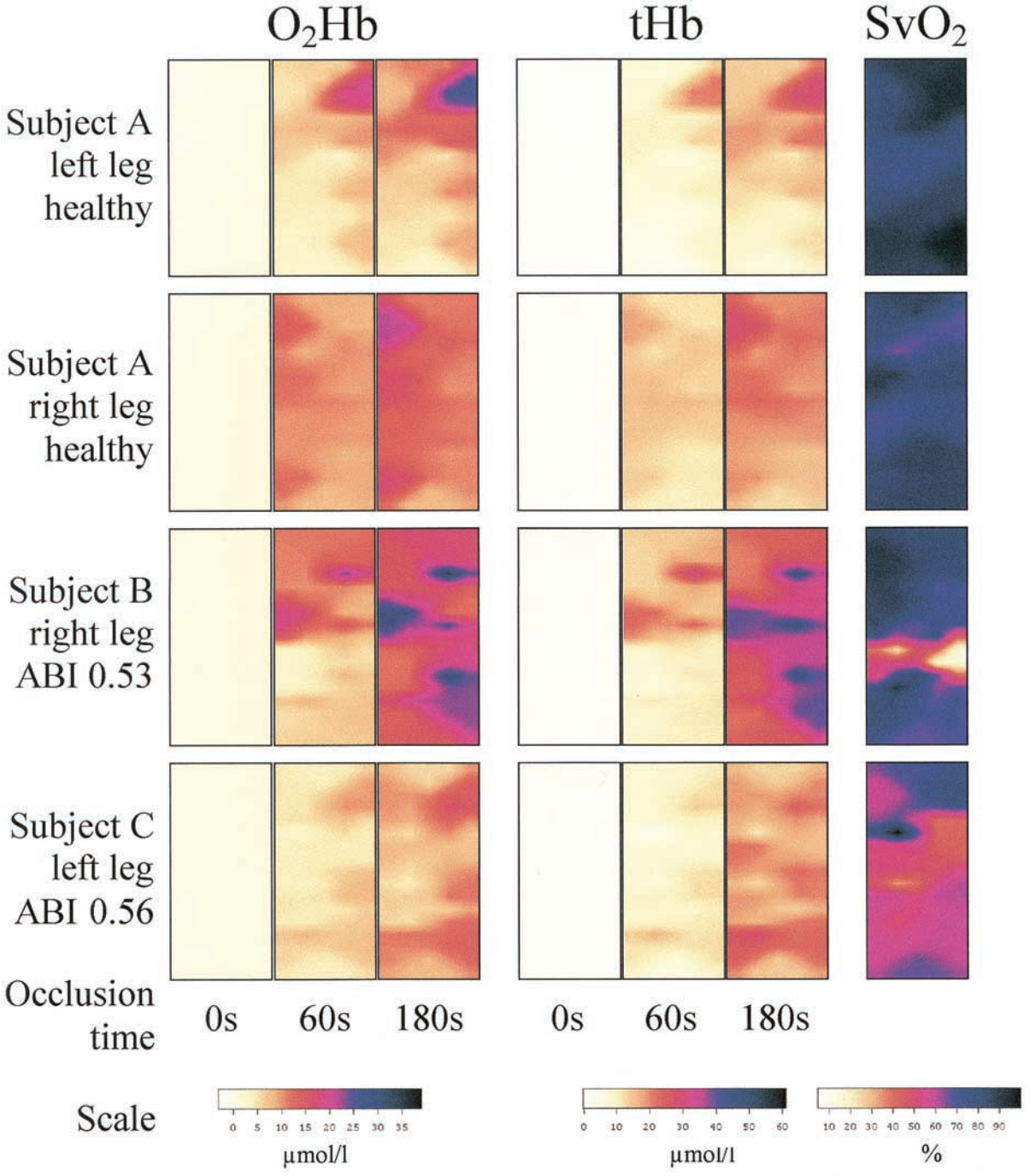

Fig 5. Color-coded maps represent view from posterior on subject lateral calf muscles during venous occlusion. Columns represent variables O_2Hb, total hemoglobin (tHb), and SvO_2, and rows correspond to a particular subject. Scale for each parameter is given at bottom. Subject A is characteristic of healthy subjects, with increase in [O_2Hb] and [tHb] is higher proximally and medially. SvO_2 is above 70% throughout maps. In subject B, localized effect with lower increase in [O_2Hb] and [tHb] and very low SvO_2 is noted, while rest of muscle is perfused and oxygenated normally. In subject C, SvO_2 drops considerably distally. *ABI,* Ankle-brachial index.

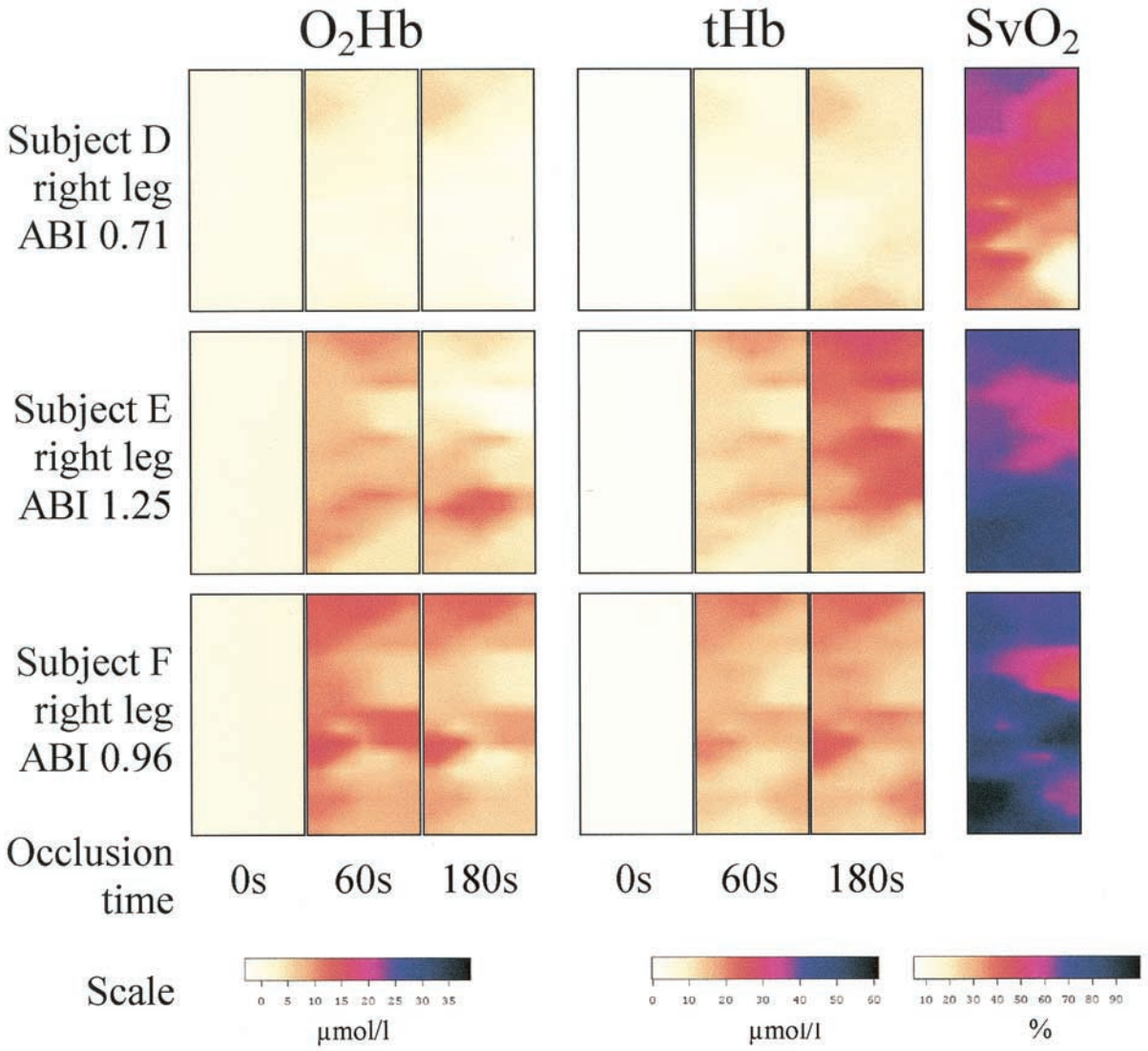

Fig 6. Maps depict phenomenon of decrease in [O_2Hb] from 60 to 180 seconds during venous occlusion. [O_2Hb] decreases despite continuous increase in total hemoglobin (tHb). Effect can be generalized (subject D) or localized (subject E). Again, maps show it is possible to identify regions of irregular hemoglobin concentration increase and SvO_2. Color-coded maps are analogous with those in Fig 5. *ABI,* Ankle-brachial index.

increasing intravascular pressure due to venous occlusion. The regions with declining [O_2Hb] overlap regions with low SvO_2. Again, the lowest SvO_2 values are not necessarily in the distal part of the calf muscle.

We would like to point out that one of the subjects (Subject E in Fig 6) with decreasing [O_2Hb] and regions with low SvO_2 had a normal ABI (ankle pressure, 176 mm Hg; brachial blood pressure, 150/90 mm Hg). Since such irregular patterns and, more important, such low SvO_2 were not observed in healthy subjects and not in all patients of similar age, they are most likely caused by vascular conditions and may be related to PVD.

Differing patterns were also observed between patients of the same age. Therefore the reason for observed differences lies within the conditions of each person rather than in age difference.

The variation within maps was significantly higher in patients compared with the healthy subjects. This reflects local irregularities in hemoglobin flow, concentration, and oxygenation, which can best be seen in Figs 5 and 6. These irregularities are larger than the normal spatial variability in healthy subjects, where higher values in the proximal and medial part were found (Fig 5).

Although these maps were generated offline, such parameters can easily be displayed in real time as venous occlusion proceeds. Inasmuch as the method is painless and requires no exertion, the maps enable identification of local irregularities in hemoglobin flow, concentration, and oxy-

genation. We believe our results reinforce the capabilities of NIRS as a promising new tool for diagnostic and follow-up purposes in treating PVD.

Limitations of the method. It is important that pressure to achieve venous occlusion is less than perfusion pressure so as not to impair arterial inflow, particularly in severe PVD, where perfusion pressure may be low. During venous occlusion [tHb] increased continuously, although at a slower rate toward the end, which demonstrates that arterial inflow was never completely blocked.

The current setup of NIRS was optimized for high spatial resolution and is sensitive to movement artifacts. During a venous occlusion protocol this poses no problem, which is confirmed by the low error of measurement during repeated measurements. For protocols involving movement, a different NIRS setup with lower spatial resolution is resistant to movement artifacts.[43]

The penetration depth of light through tissue is limited and may be influenced by adipose tissue thickness. Thus it is not possible to interrogate muscle tissue, which is more than several centimeters deep. Currently, procedures are being developed that allow three-dimensional images of tissue.

The number of subjects included in this study is limited but adequate for a feasibility study. Further studies are required to confirm the promising results and to validate the new method.

CONCLUSION

NIRS offers the unique capability of simultaneously measuring flow, concentration, and oxygenation of hemoglobin in small blood vessels, such as arterioles, capillaries, and venules, several centimeters deep in tissue. Combining NIRS with the venous occlusion method may provide a promising assessment method for PVD that is noninvasive, bedside-feasible, repeatable, and requires no exercise, which is particularly important in patients with limited locomotion capability. Significant correlations were found between ABI and increase in [O_2Hb], [tHb], hemoglobin flow, and Svo_2 at distal locations of calf muscle in the patients. The maps over a large area of calf muscle revealed localized irregularities in [O_2Hb], [tHb], and Svo_2.

As the method becomes more sophisticated and subject to further investigations, NIRS may offer great potential as a tool for diagnostic and follow-up applications, not only in patients with PVD but whenever hemoglobin flow, concentration, and oxygenation in human tissue may be impaired.

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