

## Research

**Assessment of tissue oxygen saturation during a vascular occlusion test using near-infrared spectroscopy: the role of probe spacing and measurement site studied in healthy volunteers**Rick Bezemer<sup>1,2</sup>, Alexandre Lima<sup>1</sup>, Dean Myers<sup>3</sup>, Eva Klijn<sup>1</sup>, Michal Heger<sup>4</sup>, Peter T Goedhart<sup>2</sup>, Jan Bakker<sup>1</sup> and Can Ince<sup>1,2</sup><sup>1</sup>Department of Intensive Care, Erasmus MC, University Medical Center Rotterdam, 's-Gravendijkwal 230, 3015 CE Rotterdam, the Netherlands<sup>2</sup>Department of Translational Physiology, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands<sup>3</sup>Hutchinson Technology Inc., 40 West Highland Park Drive NE, Hutchinson, MN 55350-9784, USA<sup>4</sup>Department of Experimental Surgery, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, the NetherlandsCorresponding author: Rick Bezemer, [r.bezemer@amc.uva.nl](mailto:r.bezemer@amc.uva.nl)

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*Critical Care* 2009, **13(Suppl 5):S4** (doi:10.1186/cc8002)**Abstract**

**Introduction:** To assess potential metabolic and microcirculatory alterations in critically ill patients, near-infrared spectroscopy (NIRS) has been used, in combination with a vascular occlusion test (VOT), for the non-invasive measurement of tissue oxygen saturation (StO<sub>2</sub>), oxygen consumption, and microvascular reperfusion and reactivity. The methodologies for assessing StO<sub>2</sub> during a VOT, however, are very inconsistent in the literature and, consequently, results vary from study to study, making data comparison difficult and potentially inadequate. Two major aspects concerning the inconsistent methodology are measurement site and probe spacing. To address these issues, we investigated the effects of probe spacing and measurement site using 15 mm and 25 mm probe spacings on the thenar and the forearm in healthy volunteers and quantified baseline, ischemic, reperfusion, and hyperemic VOT-derived StO<sub>2</sub> variables.

**Methods:** StO<sub>2</sub> was non-invasively measured in the forearm and thenar in eight healthy volunteers during 3-minute VOTs using two InSpectra tissue spectrometers equipped with a 15 mm probe or a 25 mm probe. VOT-derived StO<sub>2</sub> traces were analyzed for baseline, ischemic, reperfusion, and hyperemic parameters. Data were categorized into four groups: 15 mm probe on the forearm (F<sub>15mm</sub>), 25 mm probe on the forearm (F<sub>25mm</sub>), 15 mm probe on the thenar (T<sub>15mm</sub>), and 25 mm probe on the thenar (T<sub>25mm</sub>).

**Results:** Although not apparent at baseline, probe spacing and measurement site significantly influenced VOT-derived StO<sub>2</sub> variables. For F<sub>15mm</sub>, F<sub>25mm</sub>, T<sub>15mm</sub>, and T<sub>25mm</sub>, StO<sub>2</sub> downslope was  $-6.4 \pm 1.7\%/minute$ ,  $-10.0 \pm 3.2\%/minute$ ,  $-12.5 \pm 3.0\%/minute$ , and  $-36.7 \pm 4.6\%/minute$ , respectively. StO<sub>2</sub> upslope was  $105 \pm 34\%/minute$ ,  $158 \pm 55\%/minute$ ,  $226 \pm 41\%/minute$ , and  $713 \pm 101\%/minute$ , and the area under the hyperemic curve was  $7.4 \pm 3.8\%/minute$ ,  $10.1 \pm 4.9\%/minute$ ,  $12.6 \pm 4.4\%/minute$ , and  $21.2 \pm 2.7\%/minute$  in these groups, respectively. Furthermore, the

StO<sub>2</sub> parameters of the hyperemic phase of the VOT, such as the area under the curve, significantly correlated to the minimum StO<sub>2</sub> during ischemia.

**Conclusions:** NIRS measurements in combination with a VOT are measurement site-dependent and probe-dependent. Whether this dependence is anatomy-, physiology-, or perhaps technology-related remains to be elucidated. Our study also indicated that reactive hyperemia depends on the extent of ischemic insult.

**Introduction**

It is now well established that tissue oxygen utilization and regional microcirculatory oxygen transport properties are severely affected during sepsis and shock [1-9]. To assess and identify these metabolic and microcirculatory alterations non-invasively, near-infrared spectroscopy (NIRS) has recently been applied to measure the behavior of tissue oxygen saturation (StO<sub>2</sub>). Besides observation of steady-state values, a vascular occlusion test (VOT) has been introduced for the measurement of tissue oxygen consumption and of microvascular reperfusion and reactivity [9-14].

The VOT seems to be sensitive to the progress and outcome of sepsis in critical illness [9,12]. A large number of variables exist in the performance of this maneuver, however, yielding apparently conflicting results and uncertainty as to the significance of the various VOT-derived StO<sub>2</sub> parameters [11]. The analysis, interpretation, and understanding of VOT-derived StO<sub>2</sub> traces, although being widely employed in septic and trauma patients, is limited, especially for the post-occlusion phase of the VOT. Consequently, identification of

NIRS = near-infrared spectroscopy; StO<sub>2</sub> = tissue oxygen saturation; VOT = vascular occlusion test.

which  $\text{StO}_2$  parameters are most appropriate for scoring (micro)vascular reperfusion and reactivity remains to be determined. Proper characterization of VOT-derived  $\text{StO}_2$  parameters in health is hence needed to allow translation of results obtained in patients to pathophysiological phenomena.

The main problem with the interpretation of  $\text{StO}_2$  data in the literature is the diversity of methodologies used for assessing  $\text{StO}_2$  during a VOT. Results vary from study to study, making data comparison and interpretation difficult and possibly inadequate. Two major aspects regarding the inconsistent methodology are the measurement site and probe spacing (that is, the spatial separation between the illumination and detection fibers of the NIRS probe). The measurement site is important because differences may exist in the sensitivity of muscle groups and/or other anatomical structures to the VOT during health and/or pathophysiological conditions. Probe spacing, on the other hand, will determine the depth of measurement within the respective muscle group. To study the roles of both variables, we performed 3-minute VOTs in healthy volunteers and measured  $\text{StO}_2$  using 15 mm and 25 mm probe spacings on the thenar and the forearm. VOT-derived  $\text{StO}_2$  traces were quantified for baseline, ischemic, reperfusion, and hyperemic  $\text{StO}_2$  parameters. We expect these results to provide an essential frame of reference for conducting  $\text{StO}_2$  measurements in future clinical studies.

## Materials and methods

### Subjects

The study protocol was approved by the Medical Ethics Committee of the Erasmus Medical Center Rotterdam. Eight healthy volunteers (Table 1) who were not receiving any vaso-active medication were requested to refrain from consuming caffeine-containing beverages prior to the experiments. The subjects were comfortably seated in the experimental room (mean  $\pm$  standard deviation room temperature was  $21 \pm 1^\circ\text{C}$ ) 1 hour before measurements and were requested not to perform any physical labor (for example, lifting and writing).

### Near-infrared spectroscopy

$\text{StO}_2$  was continuously and non-invasively measured using two InSpectra tissue spectrometers (Model 325; Hutchinson Technology, Hutchinson, MN, USA). The spectrometers use reflectance mode probes that have a 1.5 mm optical fiber to illuminate the tissue and a 0.4 mm optical fiber to collect the backscattered light from the tissue. Two types of probes were used for this study: one with 15 mm spacing between the illumination and the collecting optical fibers, and one with 25 mm spacing. Both probes have been used in various studies with varying results [9-12].

The relative optical attenuation of the backscattered light at four wavelengths (680 nm, 720 nm, 760 nm, and 800 nm) is measured to calculate two second-derivative attenuation values, one centered at 720 nm and the other at 760 nm [15]. The ratio of the 720 nm to 760 nm second-derivative

**Table 1**

### Demographic characteristics of the studied subjects

Demographic characteristic	Mean $\pm$ standard deviation
Age (years)	28 $\pm$ 6
Gender (%)	
Female	25
Male	75
Dominant hand (%)	
Right	87.5
Left	12.5
Weight (kg)	78 $\pm$ 10
Height (cm)	178 $\pm$ 8
Tympanic temperature ( $^\circ\text{C}$ )	36.6 $\pm$ 0.5
Systolic blood pressure (mmHg)	112 $\pm$ 9
Diastolic blood pressure (mmHg)	73 $\pm$ 7
Mean arterial pressure (mmHg)	91 $\pm$ 8
Heart rate (beats/minute)	69 $\pm$ 8

values is directly extrapolated to  $\text{StO}_2$ , defined as  $[\text{HbO}_2]/([\text{Hb}] + [\text{HbO}_2])$ , via a calibration table. The calibration table relating  $\text{StO}_2$  to the second-derivative attenuation ratio is stored permanently within the NIRS device and is common to each device and probe used [15]. The NIRS devices were calibrated before the first measurement in each subject using a light-scatter calibrator.

### Vascular occlusion test

One NIRS probe was placed on the skin of the thenar eminence and another NIRS probe was placed on the lateral side of the anterior surface of the forearm for simultaneous measurement of thenar  $\text{StO}_2$  and forearm  $\text{StO}_2$  during the VOTs. Both the hand and the forearm were kept at heart level with the palms up. The subjects were instructed not to move their hand or arm, or to change their sitting position during measurements.

Baseline arterial pressure was measured using a manual sphygmomanometer. After a 3-minute stabilization period (baseline measurement), stagnant ischemia was induced for 3 minutes by rapidly inflating a pneumatic cuff (within 5 seconds), placed around the upper arm, to 50 mmHg above systolic blood pressure. The cuff was subsequently deflated (within 1 second) and  $\text{StO}_2$  measurements were continued up to 5 minutes post ischemia.

### $\text{StO}_2$ curve characteristics

$\text{StO}_2$  data from the two devices were continuously saved (one sample every 3.5 seconds) on two computers and were

retrospectively analyzed using InSpectra Analysis V3.3 software (Hutchinson Technology). The VOT-derived  $\text{StO}_2$  traces were divided into four phases: baseline, ischemia, reperfusion, and hyperemia (Figure 1).

The ischemic phase was analyzed for  $\text{StO}_2$  downslope (%/minute), minimum  $\text{StO}_2$  after 3 minutes of ischemia (%), and  $\Delta\text{StO}_2$  (%; that is, the difference between baseline and minimum  $\text{StO}_2$ ). The  $\text{StO}_2$  downslope is generally considered to reflect muscle metabolism and the minimum  $\text{StO}_2$  is considered to indicate the extent of ischemia.

The reperfusion phase was analyzed for two parameters:  $\text{StO}_2$  upslope (%/minute) and rise time (minutes), both measured over the interval from minimum  $\text{StO}_2$  to baseline  $\text{StO}_2$  (Figure 1). Although these parameters are directly related ( $\text{StO}_2$  upslope =  $\Delta\text{StO}_2/\text{Rise time}$ ), the  $\text{StO}_2$  upslope is metabolism-dependent as it is based on  $\Delta\text{StO}_2$  after a fixed time of occlusion (that is, 3 minutes), while the rise time solely represents the time required to wash out the stagnant deoxygenated blood by oxygenated arterial blood during reperfusion. These two parameters were therefore measured and analyzed separately.

The hyperemic phase of the VOT was analyzed for peak  $\text{StO}_2$  during reperfusion (%), for  $\text{StO}_2$  overshoot (that is, difference between peak  $\text{StO}_2$  and baseline  $\text{StO}_2$ ), for the area under the hyperemic curve (AUC; %·minute), and for the settling time from release of the cuff to recovery to baseline  $\text{StO}_2$  (minutes).

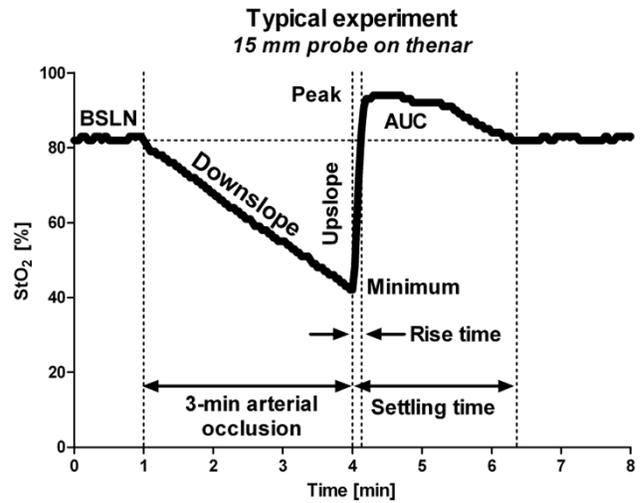
## Measurement protocol

Three measurement variables were investigated and compared for the assessment of VOT-derived  $\text{StO}_2$  parameters: dominant arm versus nondominant arm, forearm versus thenar, and superficial tissue versus deep tissue (as measured by the different probe spacings). For this purpose, four measurements were performed per subject: two on the dominant side and two on the nondominant side. Although good reproducibility of NIRS measurements during sequential VOTs has been demonstrated by Gómez and colleagues [11], the side and probes were switched after every VOT. Additionally, to avoid any effect of starting conditions, the first measurement in four subjects was performed on the dominant side with the 15 mm probe on the forearm and the 25 mm probe on the thenar, whereas in the other four subjects the first measurement was performed on the nondominant side with the 15 mm probe on the thenar and the 25 mm probe on the forearm.

## Statistical analysis

First, differences between the dominant arm and the nondominant arm were analyzed and data were subsequently categorized into four groups: 15 mm probe on the forearm ( $F_{15\text{mm}}$ ), 25 mm probe on the forearm ( $F_{25\text{mm}}$ ), 15 mm probe on the thenar ( $T_{15\text{mm}}$ ), and 25 mm probe on the thenar

**Figure 1**



Vascular occlusion test-derived tissue oxygen saturation phases and parameters. The vascular occlusion test-derived tissue oxygen saturation ( $\text{StO}_2$ ) traces were divided into four phases (baseline, ischemia, reperfusion, and hyperemia) and were analyzed for baseline  $\text{StO}_2$  (BSLN),  $\text{StO}_2$  downslope, minimum  $\text{StO}_2$ ,  $\text{StO}_2$  upslope, rise time, peak  $\text{StO}_2$ , area under the hyperemic curve (AUC), and settling time.

( $T_{25\text{mm}}$ ). Statistical analysis was performed in GraphPad Prism software (GraphPad Software, San Diego, CA, USA). Normal distribution of the data within all groups (dominant, nondominant,  $F_{15\text{mm}}$ ,  $F_{25\text{mm}}$ ,  $T_{15\text{mm}}$ , and  $T_{25\text{mm}}$ ) was confirmed for each  $\text{StO}_2$  parameter using the D'Agostino and Pearson omnibus normality test. Comparative analysis between groups was performed using analysis of variance with a Bonferroni post-hoc test. Correlation analysis was performed by Pearson's analysis for normally distributed datasets. All data are presented as the mean  $\pm$  standard deviation. Differences between groups with  $P < 0.05$  were considered statistically significant.

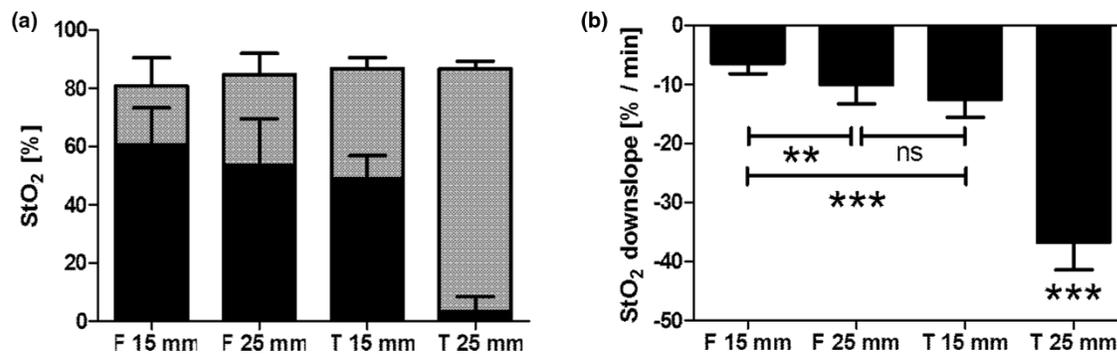
## Results

No differences between the dominant and nondominant sides were found and data could therefore be categorized into four groups:  $F_{15\text{mm}}$ ,  $F_{25\text{mm}}$ ,  $T_{15\text{mm}}$ , and  $T_{25\text{mm}}$ . Baseline  $\text{StO}_2$  was similar in all groups and independent of probe spacing and measurement site:  $81 \pm 10\%$  for  $F_{15\text{mm}}$ ,  $85 \pm 7\%$  for  $F_{25\text{mm}}$ ,  $87 \pm 4\%$  for  $T_{15\text{mm}}$ , and  $87 \pm 3\%$  for  $T_{25\text{mm}}$ . Occlusion of the upper arm by a pneumatic cuff resulted in an immediate decrease in  $\text{StO}_2$ . Release of the occlusion after 3 minutes was followed by a rapid increase in  $\text{StO}_2$  and a  $\text{StO}_2$  overshoot relative to baseline. Less than 3 minutes post-ischemia,  $\text{StO}_2$  values were restored to baseline level.

## Ischemic phase

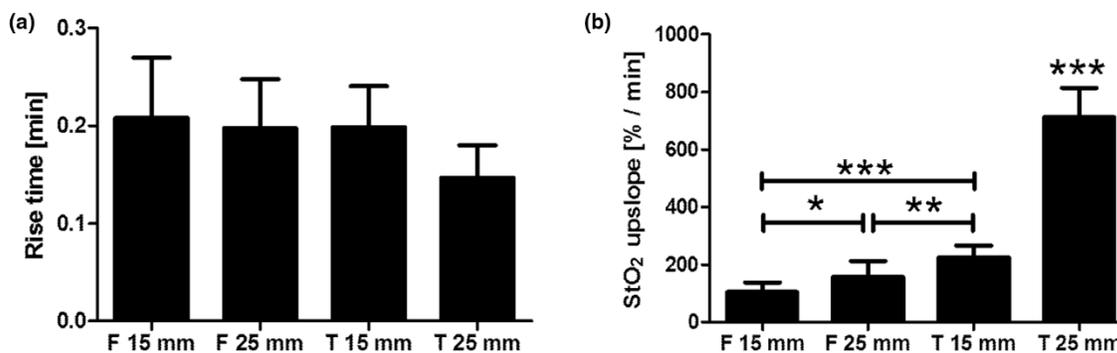
The downslopes during ischemia were measured using linear regression analysis over the linear part (that is,  $R^2 > 0.95$ ) of

Figure 2



Tissue oxygen saturation at baseline and after ischemia, and the corresponding downslopes. (a) Measured tissue oxygen saturation (StO<sub>2</sub>) baseline values and minima after 3 minutes of ischemia. (b) Corresponding StO<sub>2</sub> downslopes (right). ns = not significant ( $P > 0.05$ ), \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . F, forearm; T, thenar.

Figure 3



Measured rise times and the corresponding tissue oxygen saturation upslopes. (a) Measured rise times. (b) Corresponding tissue oxygen saturation (StO<sub>2</sub>) upslopes. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . F, forearm; T, thenar.

the StO<sub>2</sub> curve. For the first three groups (F<sub>15mm</sub>, F<sub>25mm</sub>, and T<sub>15mm</sub>), the downslopes were linear over the entire 3-minute period of ischemia. The downslopes measured in the T<sub>25mm</sub> group, in contrast, were linear over a time interval of  $2.34 \pm 0.38$  minutes. For F<sub>15mm</sub>, F<sub>25mm</sub>, T<sub>15mm</sub>, and T<sub>25mm</sub>, StO<sub>2</sub> downslopes were  $-6.4 \pm 1.7\%/minute$ ,  $-10.0 \pm 3.2\%/minute$ ,  $-12.5 \pm 3.0\%/minute$ , and  $-36.7 \pm 4.6\%/minute$ , respectively (Figure 2). Hence, during 3 minutes of ischemia, StO<sub>2</sub> decreased significantly ( $P < 0.001$ ) by  $20 \pm 5\%$ ,  $31 \pm 11\%$ ,  $38 \pm 9\%$ , and  $84 \pm 6\%$  for F<sub>15mm</sub>, F<sub>25mm</sub>, T<sub>15mm</sub>, and T<sub>25mm</sub> respectively, which resulted in a minimum StO<sub>2</sub> of  $60 \pm 13\%$ ,  $54 \pm 16\%$ ,  $49 \pm 8\%$ , and  $3 \pm 5\%$  in these groups (Figure 2).

For all groups, minimum StO<sub>2</sub> values were significantly lower than baseline values ( $P < 0.001$ ). For the downslopes and  $\Delta\text{StO}_2$  during ischemia, values for F<sub>15mm</sub> differed significantly from values for F<sub>25mm</sub> ( $P < 0.01$ ), for T<sub>15mm</sub> ( $P < 0.001$ ), and for T<sub>25mm</sub> ( $P < 0.001$ ). The minimum StO<sub>2</sub> was significantly higher in the F<sub>15mm</sub> group with respect to T<sub>15mm</sub> ( $P < 0.05$ ) and to T<sub>25mm</sub> ( $P < 0.001$ ). No significant differences between F<sub>25mm</sub> and T<sub>15mm</sub> were found for these parameters.

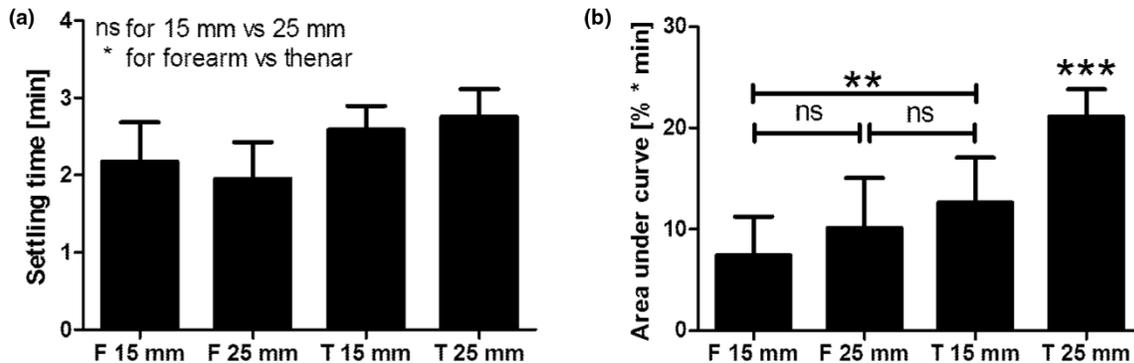
Downslopes,  $\Delta\text{StO}_2$ , and minimum StO<sub>2</sub> measured in the T<sub>25mm</sub> group were significantly different from those measured in the other groups ( $P < 0.001$ ).

#### Reperfusion phase

After release of the cuff pressure, StO<sub>2</sub> rapidly increased to (and above) baseline StO<sub>2</sub>. In F<sub>15mm</sub>, F<sub>25mm</sub>, T<sub>15mm</sub>, and T<sub>25mm</sub>, StO<sub>2</sub> rise times (that is, time from minimum StO<sub>2</sub> to baseline StO<sub>2</sub>) were  $0.208 \pm 0.062$  minutes,  $0.198 \pm 0.050$  minutes,  $0.198 \pm 0.042$  minutes, and  $0.147 \pm 0.033$  minutes, respectively. None of these results were significantly different between groups (Figure 3).

In contrast to the rise times, differences between the StO<sub>2</sub> upslopes (calculated over the same interval as the rise time) were found between groups due to differences in baselines and minima (StO<sub>2</sub> upslope = (StO<sub>2</sub> baseline - StO<sub>2</sub> minimum)/Rise time). Upslopes were  $105 \pm 34\%/minute$ ,  $158 \pm 55\%/minute$ ,  $226 \pm 41\%/minute$ , and  $713 \pm 101\%/minute$  for F<sub>15mm</sub>, F<sub>25mm</sub>, T<sub>15mm</sub>, and T<sub>25mm</sub>, respectively. The upslopes in the thenar were significantly higher than the

Figure 4



Measured settling times and the corresponding areas under the hyperemic curves. (a) Measured settling times. (b) Corresponding areas under the curves. ns = not significant ( $P > 0.05$ ), \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . F, forearm; T, thenar.

upslopes in the forearm, and the upslopes measured with the 25 mm probe were significantly higher than those measured with the 15 mm probe (Figure 3).

Although the rise time and  $\text{StO}_2$  upslope both describe (micro)vascular reperfusion following ischemia, apparently these parameters are sensitive to different variables. Hence, where the rise time is similar for the forearm and the thenar and is independent of the applied probe, the  $\text{StO}_2$  upslope depends significantly on both the muscle and the probe type.

### Hyperemic phase

Peak  $\text{StO}_2$  following release of the upper arm occlusion was  $88 \pm 7\%$ ,  $93 \pm 5\%$ ,  $95 \pm 3\%$ , and  $98 \pm 0\%$  for  $F_{15\text{mm}}$ ,  $F_{25\text{mm}}$ ,  $T_{15\text{mm}}$ , and  $T_{25\text{mm}}$ , respectively. Only the peak in the  $F_{15\text{mm}}$  group differed significantly from the thenar ( $P < 0.001$  with respect to  $T_{15\text{mm}}$  and  $T_{25\text{mm}}$ ). No significant differences were found for the  $\text{StO}_2$  overshoot (that is, peak  $\text{StO}_2$  - Baseline  $\text{StO}_2$ ):  $6.9 \pm 3.8\%$ ,  $8.6 \pm 3.7\%$ ,  $8.7 \pm 3.2\%$ , and  $11.3 \pm 2.7\%$  for  $F_{15\text{mm}}$ ,  $F_{25\text{mm}}$ ,  $T_{15\text{mm}}$ , and  $T_{25\text{mm}}$ , respectively.

The settling time, defined as the time required for the  $\text{StO}_2$  to completely restore to baseline (Figure 4), was  $2.170 \pm 0.511$  minutes,  $1.950 \pm 0.475$  minutes,  $2.588 \pm 0.306$  minutes, and  $2.755 \pm 0.360$  minutes for  $F_{15\text{mm}}$ ,  $F_{25\text{mm}}$ ,  $T_{15\text{mm}}$ , and  $T_{25\text{mm}}$ , respectively. No significant differences were found with respect to the probe spacing (that is,  $F_{15\text{mm}}$  versus  $F_{25\text{mm}}$  ( $P > 0.05$ ) and  $T_{15\text{mm}}$  versus  $T_{25\text{mm}}$  ( $P > 0.05$ )), but significant differences existed between measurement sites (that is,  $F_{15\text{mm}}$  versus  $T_{15\text{mm}}$  and  $T_{25\text{mm}}$  ( $P < 0.05$ ), and  $F_{25\text{mm}}$  versus  $T_{15\text{mm}}$  and  $T_{25\text{mm}}$  ( $P < 0.01$ )).

The AUC was  $7.4 \pm 3.8\%$ -minute,  $10.1 \pm 4.9\%$ -minute,  $12.6 \pm 4.4\%$ -minute, and  $21.2 \pm 2.7\%$ -minute for  $F_{15\text{mm}}$ ,  $F_{25\text{mm}}$ ,  $T_{15\text{mm}}$ , and  $T_{25\text{mm}}$ , respectively (Figure 4). No significant differences were found between the 15 mm probe and the 25 mm probe on the forearm. Using the 15 mm probe, the AUC in the thenar was significantly higher ( $P < 0.01$ ) than in

the forearm. AUCs measured in the  $T_{25\text{mm}}$  group were significantly higher than those measured in the other groups ( $P < 0.001$ ).

### Correlation analysis

To investigate the relationship between the extent of ischemia and the  $\text{StO}_2$  parameters of reperfusion and hyperemia, correlation analysis (Pearson's analysis) was performed for minimum  $\text{StO}_2$  versus reperfusion parameters ( $\text{StO}_2$  upslope and rise time) and hyperemic parameters (peak  $\text{StO}_2$ ,  $\text{StO}_2$  overshoot, AUC, and settling time) from combined data of  $F_{15\text{mm}}$ ,  $F_{25\text{mm}}$ , and  $T_{15\text{mm}}$ ,  $T_{25\text{mm}}$  data were excluded from the analysis because  $\text{StO}_2$  downslopes were not linear over the entire 3-minute period of ischemia, which would affect the consistency in the correlation analysis.

$\text{StO}_2$  upslope correlated significantly with minimum  $\text{StO}_2$  ( $r = 0.81$ ,  $P < 0.001$ ), while the rise time did not ( $r = 0.12$ ,  $P > 0.05$ ). The peak  $\text{StO}_2$  ( $r = 0.3$ ,  $P < 0.05$ ),  $\text{StO}_2$  overshoot ( $r = 0.44$ ,  $P < 0.001$ ), and AUC ( $r = 0.45$ ,  $P < 0.01$ ) exhibited a weak positive correlation with minimum  $\text{StO}_2$ , whereas a correlation was absent with respect to settling time ( $r = 0.16$ ,  $P > 0.05$ ).

To illustrate why the rise time and  $\text{StO}_2$  upslope behave differently in relation to the measurement site and probe spacing, two individual measurements are described in detail. One measurement was performed with the 15 mm probe on the forearm and the other with the 15 mm probe on the thenar, both with a baseline  $\text{StO}_2$  of 88%. The  $\text{StO}_2$  downslope during ischemia was  $-8\%$ /minute in the forearm and  $-16\%$ /minute in the thenar. This resulted in different  $\text{StO}_2$  minima for the two curves: 64% in the forearm and 40% in the thenar. After release of the occlusion, both curves restored back to their baseline level in 0.233 minutes. The rise times, and thus the reperfusion dynamics, for both curves were therefore equal. The  $\text{StO}_2$  upslopes, in contrast, were very different: 103%/minute in the forearm and 206%/minute

in the thenar. This suggests that the  $StO_2$  upslope does not solely reflect post-ischemia reperfusion dynamics, but is also strongly influenced by the extent of  $StO_2$  decrease during ischemia.

## Discussion

The primary finding of this study was that, although not apparent at baseline, the probe spacing and measurement site significantly influenced VOT-derived  $StO_2$  variables. The  $StO_2$  upslope in the reperfusion phase of the VOT was shown to depend on the minimum  $StO_2$  after 3 minutes of ischemia, while the rise time was not. Furthermore, the  $StO_2$  parameters of the hyperemic phase of the VOT were shown to significantly correlate to the minimum  $StO_2$  value after 3 minutes of ischemia.

Among the investigations employing a NIRS device identical to the ones used in the present study, some studies have used 15 mm probe spacing [10,11,14] while others have used 25 mm probe spacing [9,12,16-18]. In healthy volunteers, all of these studies – including ours – have shown that baseline  $StO_2$  values were similar, independent of the applied probe. The VOT-derived  $StO_2$  variables as reported in the literature, however, varied widely between the studies using a 15 mm probe and the studies using a 25 mm probe [9-12,14,16-18]. The values obtained in the present study are comparable to those obtained in the above-referenced studies. In the present study we quantitatively compared the VOT-derived  $StO_2$  variables obtained using both probes and confirmed the hypothesis that this difference in  $StO_2$  downslopes is indeed caused by the use of different probe spacings. In the thenar, the 15 mm probe provided a longer time interval of linear  $StO_2$  decay during ischemia than the 25 mm probe, which could make the estimation of the ischemic insult and muscle metabolism inaccurate and possibly inadequate when using the 25 mm probe.

The potential mechanisms by which the VOT-derived  $StO_2$  traces might be different when measured by the 15 mm or 25 mm probes could be anatomy-related, physiology-related, or even technology-related. It is well established that muscle cells consume much more oxygen per unit time compared with skin and adipose tissue. Additionally, if the ischemic stimulus is more extensive in the muscle compared to the more superficial layer of (sub)dermal tissue, the reactive hyperemia is probably also of a larger extent in the deeper, muscular, layer. In this light, the probe dependence, and thus the measurement depth dependence, of the  $StO_2$  downslope and hyperemic parameters could therefore be explained by variable relative contributions of (sub)dermal tissue and muscular tissue to the NIRS signal for the different probing depths. Another option that might explain the probe dependence of the  $StO_2$  traces, however, is that the number of photons that reach the detection fiber of the NIRS probe decreases with increasing probe spacing, which, in turn, could decrease the accuracy of the  $StO_2$  calculation. This

could be especially true at low microcirculatory oxygenation, as occurs during ischemia, where the optical absorbance of blood is much higher compared to at high oxygenation. This, however, is purely suggestive and no evidential data are present to support this speculation.

In the present article we provide a frame of reference for comparison of data measured in the thenar and forearm using the 15 mm probe and the 25 mm probe for a very broad spectrum of VOT-derived  $StO_2$  parameters; that is, baseline parameters, ischemic parameters, reperfusion parameters, and hyperemic parameters.

An important conceptual issue that is addressed in the present study is the difference between  $StO_2$  upslope and the rise time in the reperfusion phase of the VOT. First, it was shown that  $StO_2$  upslopes were different between the experimental groups while rise times were similar in these groups. Second, the correlation analysis performed on the minimum  $StO_2$  values after 3 minutes of ischemia versus the  $StO_2$  upslopes and rise times showed that the  $StO_2$  upslope correlated significantly with the minimum  $StO_2$  while the rise time did not. From a physiological point of view, the rise time represents the time it takes to wash out (or replace) the stagnantly deoxygenated blood in the measurement volume of the NIRS probe by oxygenated arterial blood. The  $StO_2$  upslope, on the other hand, has no single physiological meaning as it is the product of multiple variables, such as the baseline  $StO_2$ , minimum  $StO_2$ , and rise time. Hence, the use of rise time seems to be a more representative measure of (micro)vascular reperfusion than  $StO_2$  upslope.

Another pertinent result from the correlation analysis was the significant positive correlation between the hyperemic parameters and the minimum  $StO_2$ , indicating that the extent of hyperemia is related to the extent of ischemia. This suggests that the use of a target or threshold  $StO_2$  might be more appropriate for standardization of the hyperemic phase of the VOT. After 3 minutes of ischemia, however,  $StO_2$  decreased to a minimum of 60% in the forearm and 49% in the thenar when one uses the 15 mm probe and to 54% in the forearm and 1% in the thenar if one uses the 25 mm probe. The probe type should therefore be taken into account when one uses a defined  $StO_2$  threshold value of 30 or 40%. Moreover, the occlusion time might exceed 3 minutes when using the 15 mm probe, which could be uncomfortable for the studied subject.

Conclusively, the data from this study support the hypothesis that the NIRS measurements in combination with a VOT are measurement site-dependent and probe-dependent. The present study showed that the use of  $StO_2$  upslope is sensitive to the minimum  $StO_2$  after 3 minutes of ischemia and does not solely reflect the (micro)vascular reperfusion rate. Although the rise time seems a better measure for (micro)vascular reperfusion following ischemia, this study

could not determine whether the use of the rise time can distinguish healthy (micro)vasculature from nonhealthy (micro)vasculature. Our study also indicated that reactive hyperemia depends on the extent of ischemic insult and supports the use of a target StO<sub>2</sub> over the use of a fixed time of occlusion for a metabolism-independent analysis of (micro)vascular reactivity, whereby the type of probe should be taken into account. Whether the observed measurement site dependence and probe dependence is anatomy-related, physiology-related, or perhaps technology-related remains to be elucidated.

### Competing interests

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