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Local and remote thermoregulatory changes affect NIRS measurement in forearm muscles

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ABSTRACT

Purpose: Near Infrared Spectroscopy (NIRS) assessment in skeletal muscle is potentially affected by circulatory changes occurring in superficial tissues. Aim of this study was to separately assess the interference from skin microcirculation and large vein blood flow by investigating the effect of selective local and remote warming-induced vasodilation, respectively.

Methods: Blood volume and oxygenation changes were investigated in forearm muscles of healthy subjects in two experimental series (ES) during selective forearm (ES1, n=12) or hand warming (ES2, n=10). In ES1 the response to muscle contraction (10 s, 70% MVC) and occlusion before and after warming was also investigated while in ES2 two NIRS probes were expressly positioned over a visible vein and over a vein free area.

Results: Local warming increased the modified Beer-Lambert (BL) blood volume indicator, tHb, by 5.3 ± 3.6 μmol/L*cm to an extent comparable to post-contraction hyperemia (6.8 ± 2.9 μmol/L*cm, $p<0.01$). Remote warming increased skin blood flow at the hand and tHb at both forearm sites (on average: 5.4 ± 4.8 μmol/L*cm, $p<0.01$). Spatially-resolved (SRS) indicators of blood volume and oxygenation were not affected by any of the warming stimuli.

Conclusions: These results demonstrate for the first time that: 1) blood drained by superficial veins may affect BL measurement; 2) it is difficult to exclude veins from the measurement by simple visual inspection of cutaneous surface; 3) SRS effectively reject artifacts from superficial hemodynamic changes in both cutaneous microcirculation and large veins. These results bear implications to conditions in which thermoregulatory adjustments cannot be excluded.

KEYWORDS: Near infrared spectroscopy, muscle blood flow, muscle oxygenation, vein, skin blood flow.

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BL	Modified Beer-Lambert
ES	Experimental series
Hb	Haemoglobin
HHb	Deoxyhaemoglobin
LC	Load cell
LDF	Laser Doppler flowmetry
MVC	Maximum voluntary contraction
NIRS	Near infrared spectroscopy
O2Hb	Oxyhaemoglobin
PCH	Post-contraction hyperaemia
PVF	Presumably vein free
SBF	Skin blood flow
SII	Skin interference index
SRS	Spatially resolved spectroscopy
tHb	Total haemoglobin index
THI	Total hemoglobin index
TOI	Total oxygenation index
TRS	Time-resolved spectroscopy
VV	Visible vein
WIH	Warming-induced hyperemia

INTRODUCTION

Near infrared spectroscopy (NIRS) detects local changes in blood volume (hemoglobin concentration) and oxygenation based on the attenuation that the light backscattered by the tissues exhibits at the different wavelengths. While, generally, the interest concerns deep tissues, e.g., brain and muscle, it is clear that superficial tissues (skin, bone, and subcutaneous adipose layers) also contribute to light absorption and may affect the measurement (Canova et al. 2011; Messere and Roatta 2013; Smielewski et al. 1997; Tew et al. 2010). In particular, NIRS assessments in skeletal muscle during exercise are potentially challenged by changes occurring in the overlying cutaneous circulation, in connection with exercise-induced rise in body temperature and its regulation (Kenney and Johnson 1992).

In a recent study (Messere and Roatta 2013) we reported that superficial warming by warm air flow consistently affects standard NIRS assessment of blood volume, based on modified Beer-Lambert law. Detected changes in blood volume produced by warming-induced cutaneous dilatation were comparable to those produced by muscle dilatation occurring in a post contraction hyperaemia. The study was carried out on the biceps brachii which provides a relatively large muscle mass overlaid by thin cutaneous and subcutaneous layers. Other body areas may provide more complicated configurations with possible additional confounding factors for NIRS assessment.

For example, the forearm is composed of a large number of muscles of variable length and size and is surrounded by numerous superficial veins that drain blood from the hand. Hand circulation is mostly cutaneous and, due to its large surface, the hand represents a major site for heat dissipation (Taylor et al. 2014). Consequently thermoregulatory processes, prominently control hand blood flow and may result in considerable blood flow and volume changes in superficial forearm veins (Hirata et al. 1989; Roddie et al. 1956). We hypothesized that this could produce relevant artifacts in NIRS measurement in the forearm and that the magnitude of the disturbance could be comparable to that provoked by local warming. To our knowledge, to what extent blood in large vessels may contribute to the NIRS measurement has never been investigated.

Aim of the present work was to investigate in the forearm to what extent NIRS assessment of muscle oxygenation and blood volume can be affected by local (forearm) and remote (hand) thermoregulatory processes.

This aim is pursued by means of two different experimental series addressing the two following issues: 1) the potential influence of local cutaneous dilatation, obtained by superficial warming of the forearm; 2) the influence of remote cutaneous dilatation, obtained by selective warming of the hand.

METHODS

Two experimental series (ES1 and ES2) were conducted on two different subject groups (group 1 and group 2). In ES1 the effects of cutaneous dilatation induced by local warming and muscular dilatation produced by muscle contraction are compared. In addition, the effects of post-contraction hyperemia and of arterial occlusion are compared before and after warming in order to assess whether a maintained condition of cutaneous dilatation could change the information that is usually employed to characterize muscle contraction and to calibrate BL NIRS indices, respectively. In ES2 the potential disturbance of large superficial veins to NIRS measurement on the forearm is investigated by increasing venous return from the hand through selective hand warming.

Subjects

Twelve healthy subject were enrolled in Group 1 (9 men and 3 women; age: 33.1 ± 7.5 yr; height: 174.6 ± 8.8 cm; weight: 70.1 ± 8.4 kg; adipose tissue thickness over the muscle: 2.7 ± 0.8 mm) and 10 in Group 2 (6 men and 4 women; age: 32.1± 8.6 yr; height: 174.2 ± 9.3 cm; weight: 67.2 ± 11.1 kg; adipose tissue thickness over the muscle: 2.9 ± 0.7 mm). The study was approved by the Local Ethical Committee and all subjects gave their informed consent.

Experimental setup

Similar procedures and measurements were performed in the two series, unless otherwise stated.

NIRS assessment was performed with a continuous wave device (NIRO 200NX, Hamamatsu Photonics, Hamamatsu City, Japan) which simultaneously implements

the modified Beer–Lambert (BL) and spatially resolved spectroscopy (SRS) methods (Delpy et al. 1988; Suzuki et al. 1999; Wolf et al. 2007). BL parameters provide a measure of concentration changes in oxyhaemoglobin (O_2Hb), deoxyhaemoglobin (HHb), and total haemoglobin ($tHb = O_2Hb + HHb$) with respect to an arbitrary initial value (expressed in μmol/L*cm). Note that NIRS cannot discriminate between hemoglobin and cytoplasmatic myoglobin; therefore, all measurements actually refer to [hemoglobin + myoglobin] in the sample volume (Spires et al. 2011). However, as the myoglobin concentration is not supposed to change, all concentration changes can be attributed to blood volume variations. In order to calibrate O_2Hb and HHb, the response to arterial occlusion was recorded at the end of the experimental session. Arterial occlusion was obtained by inflating a pressure cuff around the upper-arm at 200–210 mmHg, until the O_2Hb and HHb reached a plateau level (on average after 5 min from beginning of occlusion). Concentration changes could then be expressed as percentage of the maximum excursion exhibited during arterial occlusion (Grassi et al. 2003).

By the use of several light detectors (two for NIRO 200NX) located at different distances from the light emitter, SRS technique measures the attenuation of light intensity as a function of the source-detector distance (Matcher et al. 1995). SRS provides a measure of total hemoglobin contents (THI, total hemoglobin index) and oxygenation (TOI, total oxygenation index) in tissue. THI is expressed in arbitrary units and may be reported in relative terms, e.g., as % of the control (initial) level. Instead TOI is expressed in % and represents the percentage ratio of oxygenated hemoglobin to total hemoglobin.

The NIRS probe was stuck to the forearm by double sided adhesive tape, the optodes being housed in a dark rubber holder with emitter-detectors distance of 4 cm, the two detectors being 5 mm apart. A single probe was placed over the flexor carpi radialis muscle in ES1 (Fig. 1A), while in ES2 2 two probes were placed over wrist flexor muscles: one probe was intentionally located up on a visible vein (VV) while the other was placed over an area where no visible vein could be spotted (presumably vein free, PVF), as illustrated in Fig. 1B. The possible presence of cross talk between NIRS probes was tested at the beginning of experiment and, if necessary, they were moved further apart.

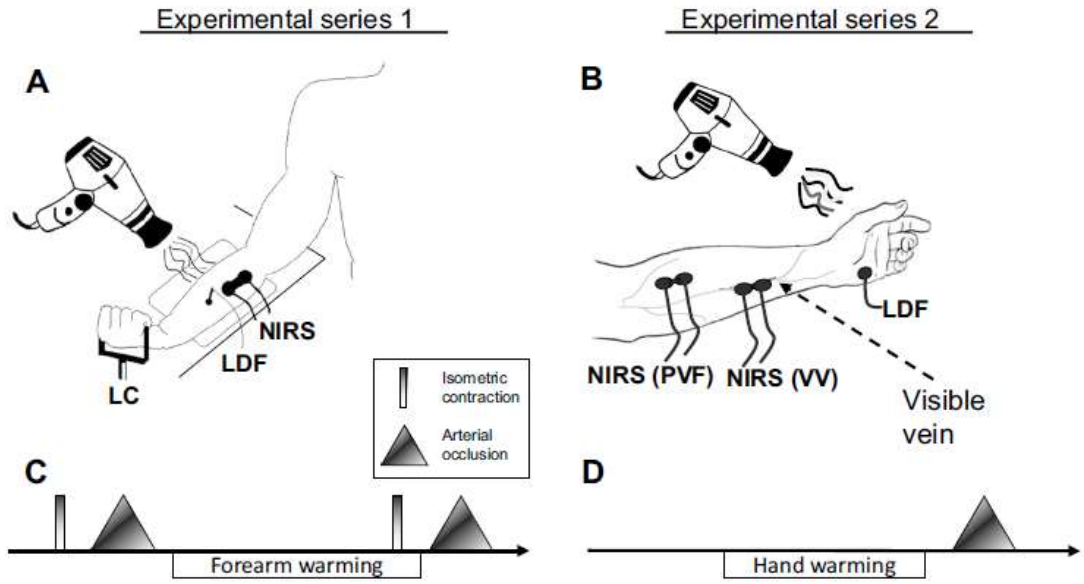

Fig. 1 Experimental setup (**a**, **b**) and protocol (**c**, **d**) of experimental series 1 (ES1) and 2 (ES2), respectively. In both cases, NIRS probes are placed over wrist flexors while warming-induced cutaneous dilatation is selectively evoked in the forearm (ES1) or in the hand (ES2). Contraction-induced muscle dilatation is also investigated in ES1 for comparison. *LC* load cell, *LDF* laser Doppler flowmetry, *NIRS* near-infrared spectroscopy, *PVF* presumably vein free, *VV* visible vein.

Adipose tissue thickness in the forearm was measured by B-mode ultrasound imaging (MyLab 25, Esaote SpA, Genoa, Italy).

Cutaneous blood flow was measured by laser Doppler flowmetry (LDF) (Periflux PF 2, Perimed KB, Stockholm, Sweden). The probe was held in place by means of a plastic holder stuck to the skin with bi-adhesive tape and was positioned on the forearm at about 1-2 cm from the NIRS probe (ES1) or on the hand palm (ES2), as indicated in Fig. 1.

Local warming of the skin was obtained by exposing the surface of the forearm (ES1) or of the palm (ES2) to warm air (t =43°C; KX2200K, Black & Decker, Towson, MD) (Messere and Roatta 2013; Ohtsuka et al. 2002; Sessler and Moayeri 1990).

Changes in cutaneous temperature were measured by a digital thermometer (Omega 450-ATH, OMEGA Engineering, Stamford, US).

Isometric wrist flexion (ES1) was performed at 70% of maximum voluntary contraction (MVC). To this purpose a visual feedback of the force intensity applied to

a force transducer (TF 02, CCt Transducers, Italy), was displayed in real time. The subject was asked to match as steadily as possible the force level indicated on a PC screen.

Experimental protocols

All measurements were performed in a quiet room with a constant ambient temperature of about 20° C. The subject sat upright on an adjustable chair with the back supported by a back rest. The forearm (right for the first and left for the second study) was positioned on a horizontal support, with an elbow angle of about 120° and the hand, oriented with the palm up.

Protocol 1

After positioning NIRS and LDF probes as described in Fig. 1A, the MVC was determined as the peak force recorded during three 3-sec lasting maximal isometric wrist flexions, separated by 2-min resting intervals. After 10 min of rest the subject performed a 10-s isometric wrist flexion at 70% MVC followed by 2 min of rest and a first arterial occlusion (about 5 min). The warming phase was started 2–3 min after the release of the occlusion, producing a gradual increase in skin blood flow up to a plateau level. Thirty seconds after reaching the plateau level the subject was asked to perform a second wrist flexion (70% MVC, 10 sec). The air flow was stopped 1 min later, lasting in total 6–9 min, depending on the time required for the SBF to reach the plateau level. A second forearm occlusion was performed at the end. The whole sequence is depicted in Fig. 1 C.

Protocol 2

After a resting period of 10 minutes, the warming procedure was performed with air flow exclusively directed to the hand. Thirty seconds after skin blood flow at the palm reached a plateau level the warm air flow was stopped. Arterial occlusion was performed two minutes later (Fig. 1D).

In two subjects the protocol was repeated with the blood flow probe positioned on the forearm rather than on the hand palm. This was aimed to verify that hand warming was not inducing cutaneous dilatation at the forearm.

In both protocols cutaneous temperature was measured in the vicinity of the NIRS probe (on the forearm in ES1, at the palm in ES2) 10 sec before the beginning and 20 sec after the end of the warming phase.

Data acquisition and analysis

The analog outputs from the NIRS device were digitally acquired along with skin blood flow and force signals with a sampling frequency of 50 Hz (CED Micro 1041, Cambridge Electronic Design, Cambridge, UK) and stored on the computer for later analysis with Spike2 software (version 6.10, Cambridge Electronic Design, UK).

The effect of warming on NIRS and skin blood flow signals was evaluated as difference of time averages over selected intervals: a 20-sec interval located just before the beginning of warming (baseline), and a 10-sec interval taken 20 to 30 s after skin blood flow reached a plateau. Changes related to post-contraction hyperemia were evaluated as differences of time averages taken over a 10-sec interval before the beginning of the contraction (baseline) and over a 2-sec interval, centered on the peak effect.

These effects are expressed in µmol/L*cm for tHb, in % of baseline for THI, in % of saturated hemoglobin for TOI and in % of the total change observed during arterial occlusion for O_2Hb and HHb. In addition, a *skin interference index* (SII) is calculated as the ratio of blood volume change detected during local warming (cutaneous dilatation) and reactive hyperemia (muscle dilatation) (Messere and Roatta 2013). This index is meant to 1) provide a quantitative indication of the dependence of NIRS parameters on skin perfusion and 2) compare the performance of the two blood volume indices, THI and tHb, whose changes are not directly comparable otherwise, since they provide a relative and an absolute measure of blood volume changes, respectively.

Statistics

Data were analyzed using the SPSS Statistics software version 20 (IBM, Armonk, NY) and are presented as mean ± standard deviation in both text and figures. Statistical comparison of the responses to contractions and warming (ES1) was performed by a one-way ANOVA for repeated measures, followed by Tukey's HSD post-hoc test, while the Student's t-test was used to assess significance of responses to

single stimuli. A 2-ways ANOVA for repeated measures with factors warming (pre/post) and probe location (VV/PVF) was used to assess changes in tHb and THI in ES2. A p-value ≤ 0.05 was required for significance.

RESULTS

The effects of local warming (ES 1)

A representative response to the isometric wrist flexion (70% MVC) and to local warming can be observed in Fig 2, while average changes are reported in the bar diagram of Fig 3. Local warming consistently increased skin blood flow (from 0.3 ± 0.1 to 2.0 ± 0.9 a.u.) and skin temperature (from 32.8 ± 1.5 to 36.4 ± 0.6 °C). A concomitant increase in blood volume was detected by tHb (5.3 ± 3.6 µmol/L*cm, $p<0.01$) but not by THI, (-0.9 ± 3.3 %). A significant increase in response to local warming was also exhibited by O_2Hb (4.6 ± 4.3 µmol/L*cm or 25.0 ± 34.7 %, $p<0.01$) and HHb (1.0 ± 1.3 µmol/L*cm or 3.7 ± 6.2 %, $p<0.05$) while TOI was not significantly affected (1.2 ± 1.8 %). Concordant changes were exhibited by the two blood volume indices during the first post-contraction hyperemia, tHb: 6.8 ± 2.9 µmol/L*cm ($p<0.01$) and THI: 10.8 ± 4.3 % ($p<0.01$). The response to the second contraction was generally larger than the first, although this reached statistical significance only for tHb (Fig. 3). From both the original tracings of Fig. 2 and the bar diagram of Fig. 3 it can be observed that changes produced by local warming in tHb are of the same order of magnitude of those produced by post contraction hyperemia. This high dependence on cutaneous circulatory changes is quantified by the SII index, which in fact results considerably larger for tHb (SII= 116.0 ± 133.3 %) than for THI (SII = -1.6 ± 32.5 %) ($p<0.05$), as illustrated in Fig. 4.

In addition, we tested whether the response to arterial occlusion was affected by the warming intervention. The occlusion-induced decrease in O_2Hb was slightly bigger after warming (pre warming: 27.3 ± 11.6; post warming: 31.1 ± 13.2 µmol/L*cm, $p< 0.05$) while the increase in HHb was not significantly affected (pre: 32.4 ± 10.8, post: 34.3 ± 12.3 µmol/L*cm).

It was also observed that the occlusion-induced increase in HHb was generally larger than the decrease in O_2Hb, yielding a net increase in tHb. Such apparent increase in

blood volume was larger during the post warming occlusion (pre: 3.2 ± 4.9 µmol/L*cm, post: 5.1 ± 4.7 µmol/L*cm, respectively, $p < 0.05$).

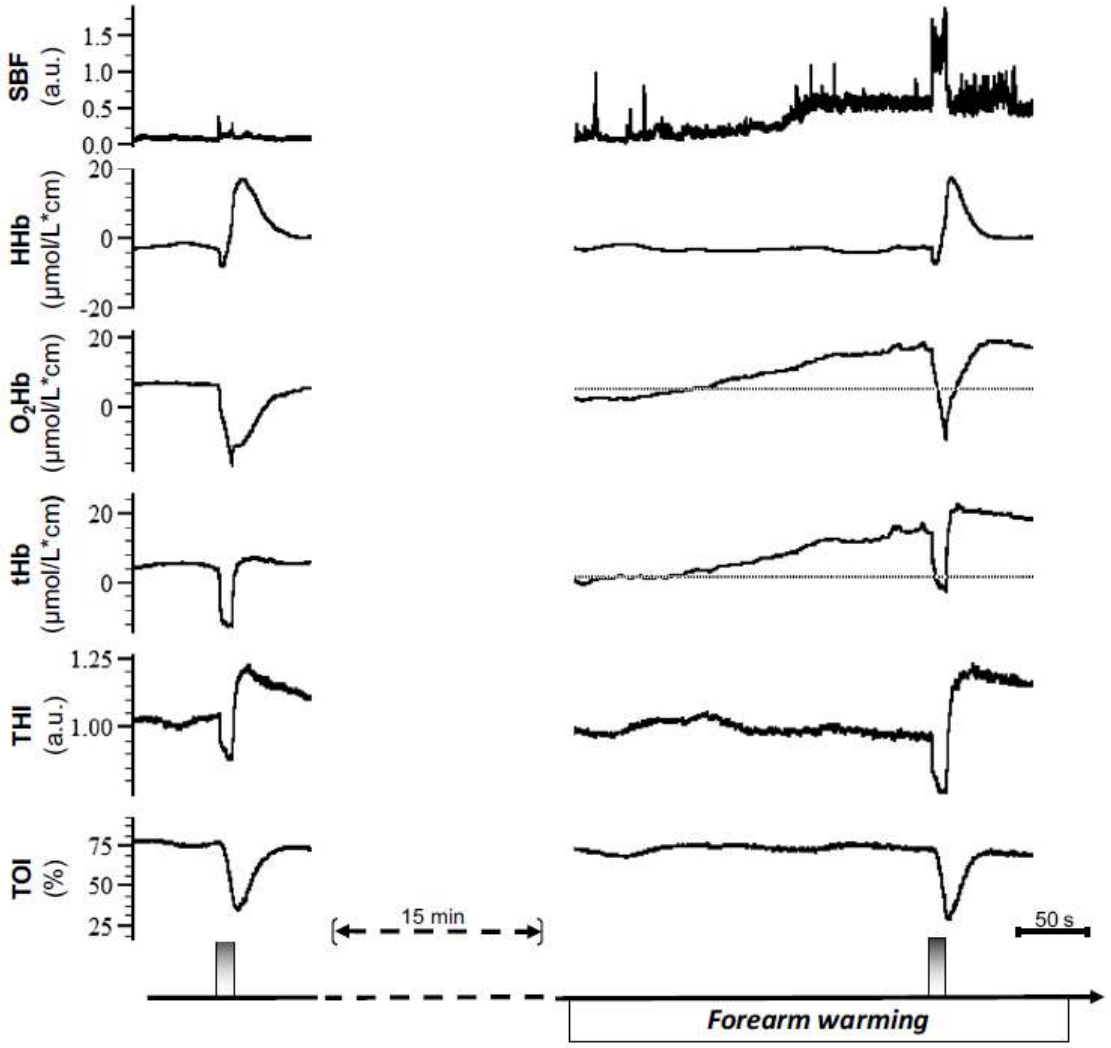

Fig. 2 Experimental series 1. Original tracings from a representative subject illustrating the response to isometric wrist flexion performed before and after local warming of the forearm by warm air flow. *SBF* skin blood flow at the forearm, *O_2Hb and HHb* changes in oxy- and deoxyhemoglobin, *tHb (=O_2Hb + HHb) and THI* changes in total Hb assessed by BL and SRS methods, respectively, *TOI* tissue oxygenation index; *bars* at the *bottom* indicate isometric contractions (70 % of MVC). *Dotted lines* indicate baseline (pre-warming) level.

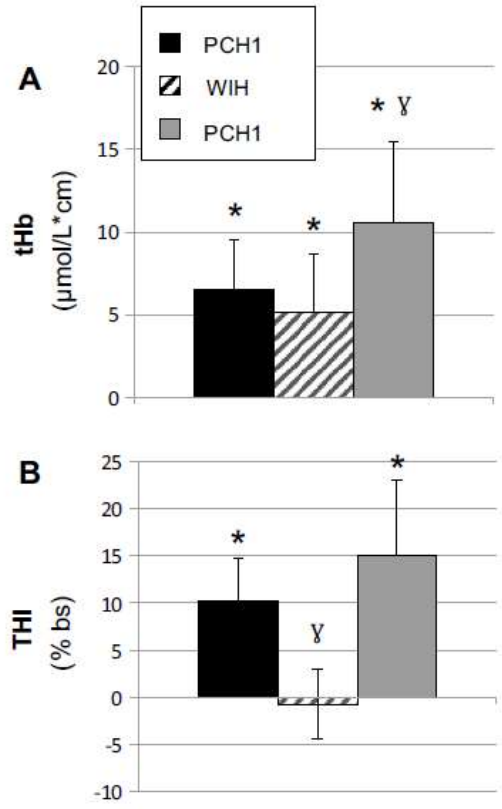

Fig. 3 Experimental series 1. Average blood volume changes detected by tHb (**a**) and THI (**b**). *PCH1* post-contraction hyperemia, *WIH* warming-induced hyperemia, *PCH2* post-contraction hyperemia after warming. *Error bars* indicate the standard deviation. n = 12. *Asterisk* significantly different from baseline ($p < 0.01$); *gamma* significantly different from the other two conditions ($p < 0.05$).

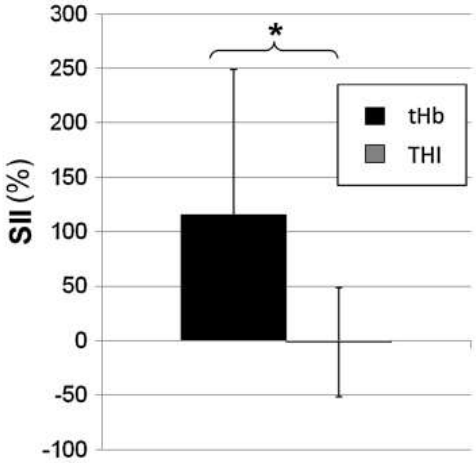

Fig. 4 Experimental series 1. Skin interference index (SII) calculated for tHb and THI. The large and significant difference observed quantifies the larger refractoriness of THI to changes in cutaneous perfusion, compared to tHb. n = 12. *$p < 0.05$.

The effects of distal warming (ES 2)

We then investigated the effect of remote distal warming (warm air flow selectively oriented to the hand) on NIRS measurement at the forearm, NIRS probes being either placed over visible veins or over skin areas apparently free of large veins (Fig. 1 B). Fig 5 shows original recordings from one subject. Hand warming produced a marked increase in skin temperature (from 25.6 ± 3.9 to 33.9± 1.3 °C) and skin blood flow at the hand (from 0.4 ± 0.3 to 2.0 ± 0.7 a.u., $p<0.01$), while leaving skin blood flow at the forearm unaffected (no change observed in 2 subjects). Changes in tHb were dependent on warming ($p<0.01$) but not on probe location (VV: 6.9 ± 5.1 µmol/L*cm; PVF: 3.9 ± 4.1 µmol/L*cm) while THI was unaffected by both warming and location (VV: -1.4 ± 11.3 %, PVF: 1.5 ± 5.0 %), see also Fig. 6.

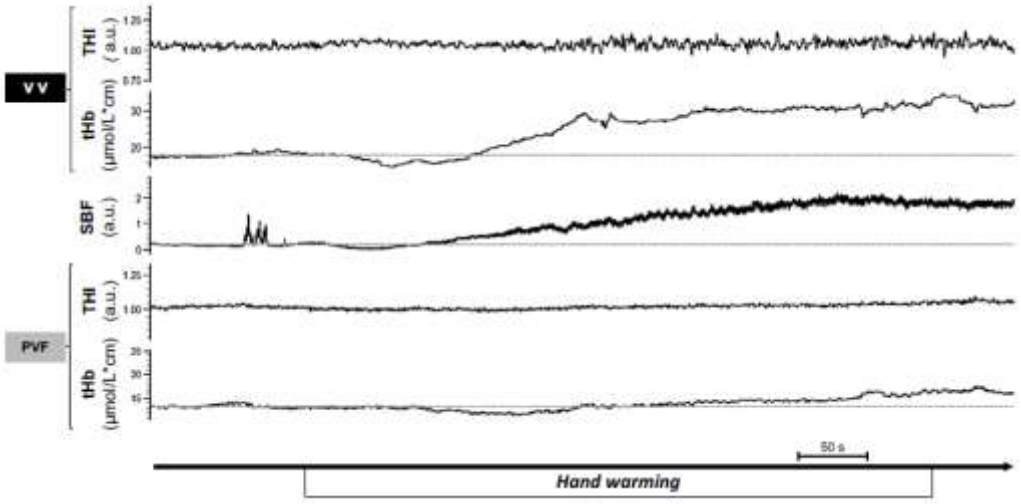

Fig. 5 Experimental series 2, original tracings from a representative subject illustrating the response to hand warming with NIRS probes placed over the wrist flexor muscles. One probe was placed over a visible vein (VV) and the other over an area where no visible vein could be spotted (PVF). SBF: skin blood flow at the hand; other abbreviations as in Fig. 2. An additional figure including the tracing of O_2Hb and HHb for both probes is available as supplementary online material.

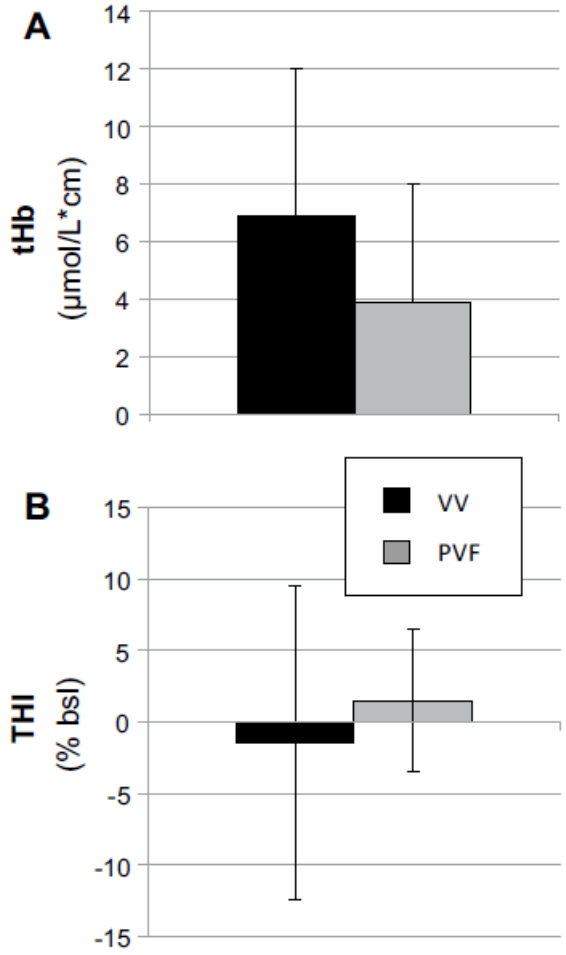

Fig. 6 Experimental series 2. Forearm blood volume changes detected by tHb (**a**) and THI (**b**) in response to selective hand warming. NIRS probes were placed up on a visible vein (VV) and over a presumably vein-free area (PVF). ANOVA evidenced a significant effect of warming but not of probe location. $n = 10$.

The reported increase in tHb was further analyzed in terms of changes of its two components O_2Hb and HHb (Fig. 7), expressed as % of the maximal change exhibited during arterial occlusion. Significant changes were only observed in O_2Hb, exhibiting a larger increase at VV (50.1 ± 42.9 %) than at PVF (9.8 ± 9.1 %) ($p<0.05$). No significant changes were exhibited by HHb (VV: 2.3 ± 21.8 %, PVF: 5.5 ± 9.3 %) and TOI (PVF: 0.2 ± 2.7 %, VV: -2.1 ± 4.7 %).

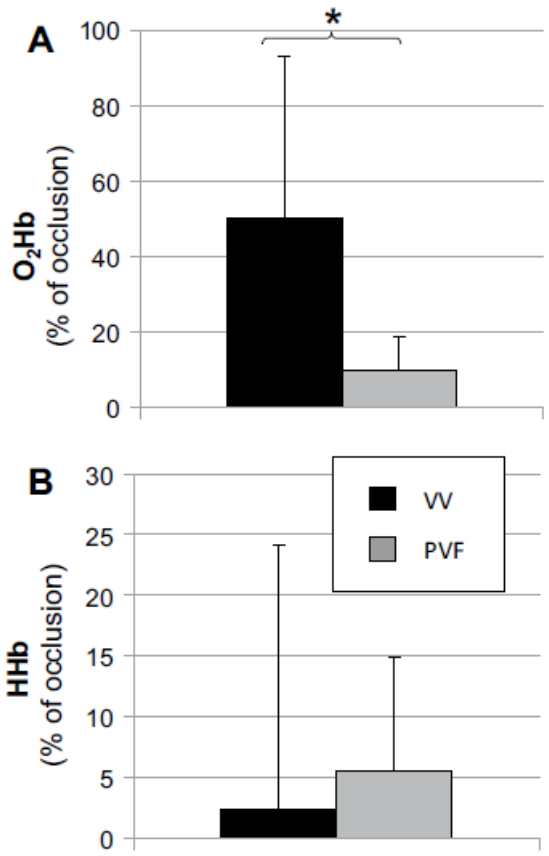

Fig. 7 Experimental series 2. Changes in O2Hb (**a**) and HHb (**b**) detected in response to selective hand warming. Changes are expressed as a percentage of the maximal variation exhibited during arterial occlusion (see "Methods"). Measurements refer to probes placed over a visible vein (VV) and over a presumably vein-free area (PVF). $n = 10$. *$p < 0.05$.

DISCUSSION

The results have shown that 1) cutaneous dilatation induced by local warming affects blood volume detection by the BL parameter tHb, confirming previous observations (Messere and Roatta 2013; Tew et al. 2010); 2) remote cutaneous dilatation induced by selective hand warming, also increases tHb, measured on the forearm, irrespective of whether the NIRS probe is expressly positioned over a visible vein or not; 3) in all conditions, the SRS blood volume index THI is not significantly affected by either local or remote increase in cutaneous circulation.

Effects of local changes in cutaneous circulation

Several studies have shown that NIRS monitoring of muscle tissues is potentially affected by changes in cutaneous circulations (Davis et al. 2006; Messere and Roatta 2013; Tew et al. 2010). In particular, we have recently shown that NIRS monitoring of the biceps brachii is affected by cutaneous dilatation of the overlying skin obtained by local warming with warm air flow (Messere and Roatta 2013). In that study it was also evidenced that the interference introduced by changes in cutaneous circulation was exclusively affecting BL parameters while SRS parameters remained largely unaffected. These observations are here confirmed in wrist flexor muscles by the results of the first experimental series: local warming produced an increase in tHb which is of the same order of magnitude as the one detected during the functional hyperemia following a 10-s, 70 % MVC contraction, while THI exhibited significant changes only in response to the latter stimulus. We are aware that both smaller and larger hyperemic responses could be produced by contractions of different intensity and duration. This type of contraction was simply chosen to provide a term of comparison for warming-induced hyperaemia. The SII index, which quantifies the sensitivity to changes in skin vs. muscle blood volume is thus considerably higher for tHb (116%) than for THI (1.6%). The comparison between the two values is even more striking than that previously reported for the biceps muscle (SII= 114% for tHb and 29% for THI). Interestingly, similar artifacts produced by thermoregulatory changes in skin blood flow have been recently reported on BL NIRS parameters while monitoring cerebral haemodynamics (Miyazawa et al. 2013). The authors evoked increase and decrease in forehead skin blood flow by exercise and facial cooling, respectively, and observed parallel changes in all BL parameters (tHB, O_2Hb and HHb) while the SRS parameter TOI remained unchanged.

We previously observed that although basal tHb levels were affected by local warming, the hyperemic response to muscle contraction was only translated upwards but not affected in magnitude (Messere and Roatta 2013). At difference from this previous study on the biceps muscle, the hyperemic response presently observed appears to be larger after local warming: changes in both tHb and THI increased by about 50% after warming, although only tHb reached statistical significance. However, in the present study the second muscle contraction was also preceded by an artery occlusion. Although ischemic preconditioning has been shown to increase

functional hyperemia in cardiac muscle (Gattullo et al. 1999) a relevance of this mechanism in skeletal muscle function has not been documented. Thus, no univocal conclusion can be drawn as for the mechanism responsible for the increased tHb response to muscle contraction on the basis of the present data.

The possibility that the response to arterial occlusion, which is used to calibrate O_2Hb and HHb changes in NIRS studies, could also be affected by cutaneous circulation was also investigated here by comparing the responses obtained before and after local warming (ES1 protocol in Fig. 1C). The results indicate that the decrease exhibited by O_2Hb during arterial occlusion is significantly augmented after warming. This effect can be explained by the larger amount of blood volume available in the forearm after warming. One would also expect to observe a corresponding increase in the HHb variation, which however did not reach statistical significance. This discrepancy, which gives rise to a net increase in the total blood volume, tHb, has already been observed and explained as a possible redistribution of the blood among the different tissues during the occlusion (Cheatle et al. 1991; Kragelj et al. 2000; Praagman et al. 2003; van Beekvelt et al. 2002).

Although not all effects are as yet univocally explained it is clear that changes in cutaneous microcirculation, as produced by local warming, affect basal values BL parameters as well as the reference values that are currently employed for their normalization.

Effect of increased flow in large superficial veins

The forearm is peculiar for the amount of large superficial veins that drain blood from the hand, a relevant heat sink for the human body (Taylor et al. 2014). In fact blood flow to and from the hand is known to vary extensively according to thermoregulatory needs (Yamazaki and Sone 2006). Accordingly, substantial changes in venous return take place through superficial veins of the forearm in response to central or peripheral thermal challenges (Hirata et al. 1989). The presence of large veins in the NIRS sample volume is generally overlooked based on the consideration that the contained blood would completely absorb the light passing through the vessel and would not contribute to light backscattered to the optode (McCully and Hamaoka 2000). However, a variation of blood volume in the vein, possibly related to a variation in venous return, would obviously modulate the intensity of the backscattered light and

the blood volume measurement. Surprisingly, the potential disturbance introduced by superficial veins on NIRS monitoring has never been investigated before, to our knowledge. Results from ES2 clearly showed that selective increase in blood flow to the hand is detected in terms of increased tHb at the forearm. Although this increase tended to be higher when the probe was intentionally placed over a large visible vein, no significant difference was detected between responses collected from VV and PVF. This may be due to the fact that part of the blood drained from the hand is carried by small veins or by veins not clearly visible from the surface. It must be also considered that in many subjects even the largest forearm veins are hardy visible and the possibility that they lie under the NIRS probe thereby affecting tHb cannot be realistically excluded. Remarkably, THI was in both cases (VV and PVF) not significantly affected by hand warming, suggesting that the SRS methodology is effective in rejecting also this type of disturbance and thus provides indicators of hemodynamic changes that, compared to BL, more specifically concern deep (muscle) tissues.

These observations bear important methodological implications: 1) changes in blood volume occurring in large superficial veins as may occur during postural changes as well as during thermoregulatory adjustments can introduce artifacts in NIRS monitoring that may be erroneously interpreted as local changes in tissue Hb concentration; 2) such effect can be reduced but not completely excluded (not even in slim subjects presenting a clearly visible venous network) by placing the probe apart from visible veins on the basis of visual inspection; 3) SRS parameters should be used whenever changes in blood content in these vessels are expected.

Oxygenation in muscle tissue

The increases observed in O_2Hb could be erroneously interpreted in terms of increased oxygenation in forearm muscles, particularly in the case of remote warming (ES2) where the increases in O_2Hb is accompanied by unchanged HHb. In fact, there is no reason why blood flow should increase in forearm resting muscle during hand warming. On the contrary, a decrease was suggested to take place, in response to contralateral hand warming (Astrup et al. 1988). On the other hand, the large amount of skin blood flow that returns from the hand through forearm superficial veins has characteristics very similar to the arterial blood, to the extent that venous blood

sampling from heated hand has been employed as a possible alternative to arterial blood sampling (Brooks et al. 1989). Thus the increase in O_2Hb and not in HHb observed in ES2 fits with the idea of increased amount of oxygenated blood in the venous return through forearm veins, during hand warming.

These data confirm previous observations based on non-SRS NIRS of increased oxygenation in response to both local (Buono et al. 2005) and whole-body warming (Davis et al. 2006). In contrast, Koga et al (Koga et al. 2015) reported a significant decrease in tissue oxygenation in response to whole-body heating, by using a time-resolved NIRS. While a physiological explanation for this effect was not provided, the disagreement could be related to differences in experimental conditions, namely a relatively low (3-fold) increase in cutaneous blood flow elicited by the warming procedure and the use of time-resolved instead of continuous wave spectroscopy (discussed below) (Koga et al. 2015).

Remarkably, in the present study, the SRS TOI remained insensitive to both local and remote warming, while being normally responsive to muscle contraction (Fig. 2). This emphasizes the concept of high refractoriness of both blood volume and oxygenation SRS indices from hemodynamic changes occurring in superficial tissue layers.

Dependence of NIRS monitoring on cutaneous circulatory changes

Changes in [Hb] assessed thorough standard BL methodology are based on intensity changes in the light back-scattered by the tissue with no distinction between superficial and deep layers. Several report in the literature have now clearly shown that changes in cutaneous circulation affect BL measurements that aim at monitoring blood volume and oxygenation changes in deeper tissue layers e.g., muscle and brain (Al-Rawi et al. 2001; Canova et al. 2011; Messere and Roatta 2013; Miyazawa et al. 2013). The SRS methodology is based on detecting the back-scattered light at multiple distances from the emitter and on assessing the slope of light attenuation with distance. This differenctial operation virtually eliminates the common components in the detected signals which originate from superficial layers (Delpy and Cope 1997; Quaresima and Ferrari 2009). This theoretical basis explains several experimental reports of relative independence of SRS parameters from cutaneous circulatory changes (Al-Rawi et al. 2001; Canova et al. 2011; Kirkpatrick et al. 1998; Messere and Roatta 2013). The present data provide further support to this notion.

Interestingly, also time-resolved spectroscopy (TRS) (Oda et al. 2000; Ohmae et al. 2006) appears to have a greater sensitivity to deep rather than superficial tissues (Koga et al. 2015; Sato et al. 2007). For instance, Sato et al (Sato et al. 2007) showed that TRS NIRS was not affected by clamping the external carotid artery in neuro-surgical patients. More difficult to interpret are the results of Koga et al. who compared the changes in standard BL and TRS NIRS parameters produced by whole-body warming in forearm muscles (Koga et al. 2015). They showed that increased blood volume associated with cutaneous dilatation was detected by BL and not TRS parameters. However, TRS NIRS also detected an unexplained increase in HHb and a decrease in tissue oxygenation. A direct comparison between SRS and TRS techniques could help in the understanding of advantages and limitations of these techniques.

Methodological considerations

NIRS monitoring during warming could be affected by changes in muscle blood flow as has been shown to occur in response to a 4-°C increase in muscle temperature (Heinonen et al. 2011). Although we did not measure muscle blood flow, the increase in muscle temperature was considered very unlikely to occur in the present experimental conditions, in which warm air flow is directed towards a limited body surface for a short period of time (Messere and Roatta 2013). In fact, warm air flow has been frequently employed to induce marked and stable vasodilatation selectively limited to the cutaneous tissue layer and to the exposed body surface (Barcroft and Edholm 1943; Hodges et al. 2009; Johnson and Kellogg 2010; Kellogg et al. 1995; Messere and Roatta 2013; Minson et al. 2002). In particular, we here verified in two subjects that hand warming did not affect cutaneous blood flow on the forearm. It must be emphasized that this procedure simulates thermoregulatory adjustments comparable to those occurring in several physiological situations such as exercise and hyperthermia (Davis et al. 2006; Fritzsche and Coyle 2000; Kellogg et al. 1991; Yamazaki and Sone 2003, 2006).

In addition, the effects of local and remote warming, that have been here separately investigated, concomitantly occur in response to a rise in body temperature and are thus expected to provide additive disturbance to the NIRS measurement, particularly

in terms of an increase in O_2Hb (although HHb was also significantly affected in ES1).

During exercise, this hyperemia of the superficial layers will overlap with the functional hyperemia in skeletal muscle. It will thus be impossible to discern the individual contribution of these two components to blood volume and oxygenation changes detected by BL NIRS parameters. In this condition neglecting the contribution from skin and superficial veins will lead to an over-estimation of the increase, or to a under-estimation of the decrease of BL parameters, the effect being comparatively smaller for HHB than for O_2Hb and tHb. This consideration supports the concept of preferentially relying on HHb for assessing muscle de-oxygenation during exercise (Grassi et al. 2003), when SRS is not available.

Adipose tissue has also been reported to affect NIRS parameters (McCully and Hamaoka 2000). A B-mode ultrasound device was here employed to measure the subcutaneous adipose tissue thickness at the location where the NIRS probe unit was placed. Considering that the fat thickness was relatively low, and the penetration depth of the NIRS signal is almost half of the emitter-detector separation (4 cm in the optical probe of the NIRO-200NX), its contribution to the measurement was probably negligible for BL parameters and even more so for SRS that reduces the contribution of superficial layers (Felici et al. 2009).

Conclusions

Confounding factors affecting NIRS monitoring of (intendedly) muscle tissue include not only circulatory changes in the overlying cutaneous tissue but also changes in blood flow of superficial veins that happen to cross the sample volume of the NIRS probe. These factors should be taken into account whenever thermoregulatory reactions are expected and the investigated body areas are characterized by a high density of superficial veins, as in the forearm. Spatially-resolved spectroscopy appears to be an effective methodology to reject interference from both cutaneous tissue and superficial veins.

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Electronic supplementary material

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