

NOTE

Mapping human skeletal muscle perforator vessels using a quantum well infrared photodetector (QWIP) might explain the variability of NIRS and LDF measurements

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Abstract

Near-infrared spectroscopy (NIRS) and laser Doppler flowmetry (LDF) have become the techniques of choice allowing the non-invasive study of local human skeletal muscle metabolism and blood perfusion on a small tissue volume (a few cm³). However, it has been shown that both NIRS and LDF measurements may show a large spatial variability depending on the position of the optodes over the investigated muscle. This variability may be due to local morphologic and/or metabolic characteristics of the muscle and makes the data interpretation and comparison difficult. In the present work, we use a third method to investigate this problem which permits fast, non-invasive mapping of the intramuscular vessel distribution in the human *vastus lateralis* muscle. This method uses an advanced, passive, infrared imaging sensor called a QWIP (quantum well infrared photodetector). We demonstrate, using a recovery-enhanced infrared imaging technique, that there is a significant presence of perforator vessels in the region of interest of $\sim 30 \times 18$ cm (the number of vessels being: 14, 9, 8, 33, 17 and 18 for each subject, respectively). The presence of these vessels makes the skeletal muscle highly inhomogeneous, and may explain the observed NIRS and LDF spatial variability. We conclude that accurate comparison of the metabolic activity of two different muscle regions is not possible without reliable maps of vascular 'singularities' such as the perforator vessels, and that the QWIP-based imaging system is one method to obtain this information.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Near-infrared spectroscopy (NIRS) and laser Doppler flowmetry (LDF) techniques have acquired a great momentum in the past few years (Delpy and Cope 1997, Leahy *et al* 1999) because they allow the non-invasive study of local tissue metabolism and blood perfusion in many different human tissues such as, e.g., skeletal muscle (Ferrari *et al* 1997), brain (Obrig and Villringer 2003) or bone marrow (Binzoni *et al* 2003). In particular, these techniques allow one to follow localized (i.e. on a tissue volume of few cm³) physiological phenomena where whole body metabolic measurements would be inappropriate, e.g. as in the case of localized intramuscular temperature changes (Binzoni *et al* 2002). It appears that in these particular cases optical techniques represent the only applicable non-invasive approach. However, it has also been demonstrated that both NIRS and LDF measurements may show a large spatial variability, depending on the position of the optodes on the investigated muscle (Miura *et al* 2001, Quaresima *et al* 2001, Binzoni *et al* 2003).

Changes in metabolic activity are usually estimated by NIRS by directly measuring oxy- (HbO₂) and deoxyhaemoglobin (Hb) (Delpy and Cope 1997). Unfortunately, tissue haemoglobin saturation depends not only on metabolic activity but also on blood perfusion. The relationship existing between tissue metabolism and blood perfusion is not simple (Cerretelli and Binzoni 1997). Thus, it may be very difficult to distinguish real metabolic changes from variations of the NIRS signals induced by simple blood perfusion changes. As a consequence, it appears that in future simultaneous measurements with both NIRS and LDF may become mandatory if one wants to improve one's understanding of tissue energy metabolism.

Even if the combination of NIRS and LDF may improve the measurement technique, there still remains an unexplained variability related to both techniques. This is especially true when dealing with human skeletal muscle measurements. It has been observed that in the human *gastrocnemius medialis* muscle, the changes in haemoglobin oxygen saturation (defined in this case as $\Delta\text{HbO}_2 - \Delta\text{Hb}$) and blood volume ($\Delta\text{HbO}_2 + \Delta\text{Hb}$) changes determined by NIRS, may depend on the position of the investigated anatomical region (Miura *et al* 2001). This indicates that NIRS is very sensitive to metabolic and/or morphological characteristics of muscle tissue. These characteristics greatly influence local haemoglobin saturation, at rest or during exercise and thereby the consistency of the NIRS measurements. This effect has been clearly demonstrated on human quadriceps muscle by Quaresima *et al* (2001). Further studies have demonstrated that there is a linear correlation between the NIRS parameters (HbO₂ - Hb and HbO₂ + Hb) and the muscle pennation angle and/or the fascicle length (Miura *et al* 2003). Thus, the morphological structure of the muscle seems to explain part of the differences observed during exercise. In this case the authors hypothesized that the observed differences arose from different intramuscular pressures, due to the different muscular morphologies, which influence the blood perfusion and volume and thus HbO₂ - Hb.

On the other hand, the studies of Quaresima *et al* (2001) have shown that NIRS measured O₂ consumption is not uniform in the human quadriceps (*vastus lateralis*, *rectus femoris*) at rest. In this study the O₂ consumption measurements were performed using the standard cuff occlusion method (De Blasi *et al* 1993), so the variability will not be due to the difference in blood perfusion, since, due to the occlusion, the velocity is zero by definition in all the parts of the muscle. Thus, in this instance the spatial variation of the NIRS parameters must be explained by other factors, such as the muscle fibre type or by some more macroscopic differences in the vascular structure. LDF measurements on human forearm (Binzoni *et al* 2003) at large optode spacing (2 cm) have shown that slightly moving one of the two NIRS optodes by a few millimetres results in abrupt changes in the measured blood velocity,

e.g., from 9.99 mm s^{-1} to 5.18 mm s^{-1} . This abrupt change might be explained by the presence of a localized increased vascularization, i.e. a low blood velocity may be caused by the presence of a large blood vessel, since for a given blood flux, the larger the diameter the lower the velocity.

The presence of such vessels and the fact that they may change the homogeneity of the muscle was indeed proposed more than 100 years ago and the first map of what are usually called human ‘perforator vessels’ was made in 1889 on a human cadaver by Manchot (1889). Today, the precise localization of perforator vessels represents an important preoperative task in reconstructive plastic surgery and several techniques have been proposed to allow their non-invasive detection such as the use of the Doppler ultrasound (Hallock 2003), thermographic mapping (Itoh and Arai 1995) or magnetic resonance imaging (Ahn 1994).

Thus, to map the perforator vessels and clarify the problem of the NIRS and LDF variability we have chosen to use the infrared imaging technique. We employed an infrared imaging system (BioScanIR, Omnicorder Technologies, Inc.) with much higher sensitivity, speed and data processing capabilities as compared to the system employed by Itoh and Arai (1995). This decision was dictated by the fact that Doppler ultrasound and MRI only permit one to observe relatively large vessels and may not be capable of detecting local capillaries or arteriolar/venular density changes, which may also affect NIRS and LDF measurements. In practice, infrared imaging allows one to observe the presence of vessels by detecting the heat transported by the blood and hence by conduction/convection reaching the surface of the observed body region.

Thus, the aim of the present work was to: (1) choose the human *vastus lateralis* muscle as a model and build a surface map, using an infrared imaging system, showing the distribution of vessels that influence the homogeneity of the vascular bed (and thus possibly cause NIRS or LDF metabolic/perfusion measurement artefacts); (2) study the inter-subject variability of these maps; (3) discuss the possible influence of these vessel distributions on the different NIRS or LDF parameters.

2. Methods

The perforator vessels were detected by means of the recovery-enhanced infrared thermography method proposed by Itoh and Arai (1995).

2.1. Infrared imaging

The infrared images were acquired using a BioScanIR System (Omnicorder Technologies Ltd., E Setauket, NY, USA). The system utilizes a 256×256 single-band QWIP sensor. The QWIP is a narrow band detector operating in the long wave (8–10 μm) region. This wavelength is optimal for detecting emissions for biological applications. Human tissue is 98% emissive at this wavelength with only 2% of the radiation coming from environmental sources and reflecting from the surface of the skin. This is an important point because ‘classical’ large bandwidth detectors may detect up to 30% reflected light and thus weak signals may be masked by unwanted sources of IR radiation. The QWIP sensitivity is less than $0.009 \text{ }^\circ\text{C}$ between pixels and with a temporal resolution of up to 400 Hz. This sensitivity cannot be reached with classical infrared systems. This degree of sensitivity requires cooling the detector to the cryogenic temperature of 60 K with a specially designed Sterling Cooler pump unit. The QWIP spatial resolution is 40 μm , with more than a 99.5% yield of operating pixels. The output intensity range for each pixel was encoded over 14 bits.

The camera is equipped with two combined multiple element f8.0/20 mm and f2.0/48 mm, germanium lenses. Data from the camera head are collected, stored and analysed on a specially configured PC equipped with a high-speed frame grabber, dual 1.6 GHz processors, 4 GB of RAM and three 120 GB hard drives. This configuration allows one to acquire, display and analyse thermographic images in real time. The raw and/or processed data can be stored in real time to the hard drives for later analysis or reprocessing. The system includes a suite of proprietary processing software and an integrated patient data management system.

The camera calibration (pixel intensity working range) and the image calibration in absolute temperature values are performed using an integrated infrared radiation source, with a temperature accuracy of ± 0.008 °C, temperature uniformity of 0.01 °C and emissivity of 0.97 ± 0.01 over the entire emitter. Calibration of the IR images is performed using a two-point linear fitting procedure, correlating temperature and pixel intensity.

2.2. Protocol

The present investigation was performed on six healthy male subjects (35.8 ± 5.5 years). The subject was asked to lie supine on the examination table with the back of the bed positioned at 30°. The infrared camera position allowed visualization of the thighs of subject and in particular the right *vastus lateralis* muscle. The images were displayed in real time (100 frames per second) on the computer screen during the whole experimental period. Using the image acquisition software controlling the camera, one image every 15 s was stored on the hard disk for later offline analysis. The protocol was: (1) start the image acquisition and saving procedure; (2) a cooling bag (Ortlieb Sportartikel GmbH, Heilsbronn, Germany) filled with water mixed with ice was placed over the *vastus lateralis* muscle for 20 s. The bag was flexible enough to uniformly cover the region of interest, eliminating any possible artefactual 'hot' lines due to fold in the bag and poor contact of the cooling medium with the skin. (3) After 20 s the cooling bag was removed from the leg and the appearance of the perforator vessels was followed visually in real time on the computer screen. The measurements were stopped within 5 min for all the subjects when no new perforators were appearing on the screen.

3. Results

In figure 1 are shown a series of images representing the temperature time evolution just after the ice bag was removed from the *vastus lateralis* muscle of one particular subject. The head of the subject (supine) is on the left-hand side of the image. The images are presented at 15 s intervals. The time increases from the left to the right and from the top to the bottom, so the whole array represents 5 min recovery. The temperature is represented by the NASA 'jet' colourmap on the colourbar. The deep blue colour means that the temperature is ≤ 24 °C and the red-brown colour means that the temperature is ≥ 33 °C. The position where the ice bag was placed appears as a deep blue region in the first image of the sequence. This region is over the right *vastus lateralis* muscle. During the rewarming (recovery) period, with the room at ambient temperature, the perforator vessels appear as irregular spots with a higher temperature than the surrounding background, i.e. the region where the perforator vessel is present heats faster than the background. The vessel size has certainly an influence on the spot size. It must be noted that a large vessel may carry more heat than a small one and thus induce larger temperature changes in its neighbourhood.

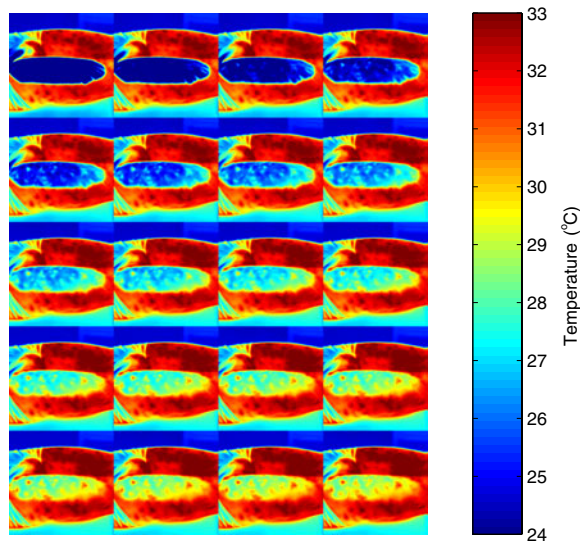


Figure 1. Temperature image time series for one subject during the rewarming period with the room at ambient temperature. The time interval between two adjacent images is 15 s. The time increases from the left to the right and from the top to the bottom. The deep blue region (see electronic version) on the first image covers the right *vastus lateralis* muscle (subject supine, head on the left-hand side of the image). It is possible to see also the left leg and part of the groin region.

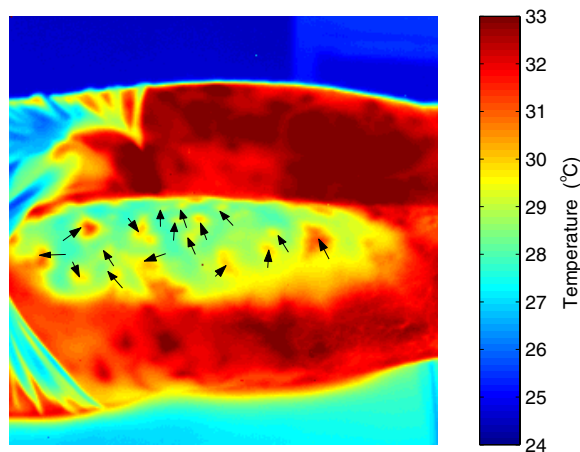


Figure 2. This figure shows the second image appearing in the last line of figure 2 and corresponds also to the bottom-right image in figure 3. The vessels are in this case marked by an arrow. The localization of the vessels is easily made by following an image time sequence.

Figure 2 shows the second image of the last row from figure 1. The arrows indicate the positions of the main perforator vessels.

Figure 3 summarizes the results of the present study. Each image represents one subject and the bottom-right image corresponds to the subject shown in figure 1. The white and black representation of the temperature in this case gives the anatomical landmarks, whilst the superficial positions of the perforator vessels appear as coloured dots over the region of interest

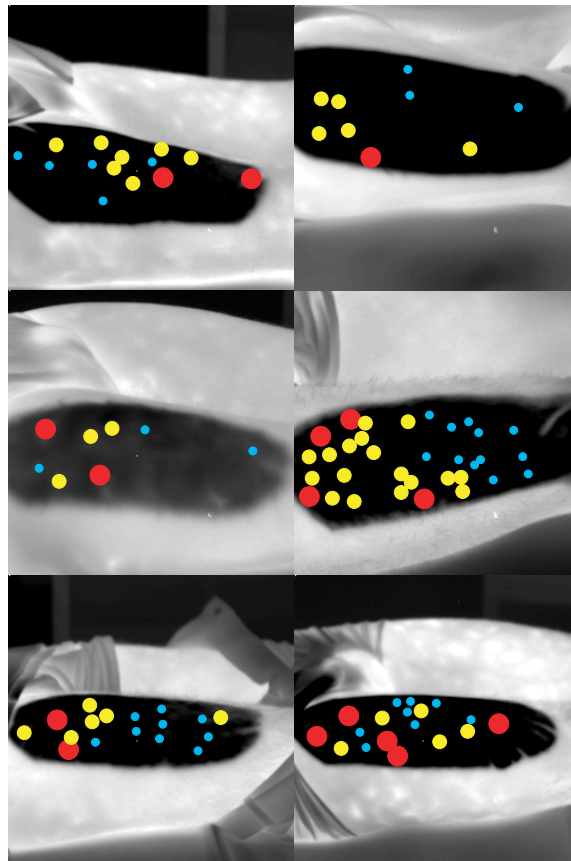


Figure 3. The superficial positions of the perforator vessels for the six subjects are represented as coloured dots. Each image represents a different subject. Large dots (red) indicate that these perforators are the first to appear during the rewarming, the medium (yellow) the seconds and the smallest (cyan) are the last. The black region is the region of interest previously cooled by the ice bag.

(black region). The black region represents the surface cooled by means of the ice bag (the length is ~ 30 cm and width ~ 18 cm in all six figures). It is important to note the variability of the distribution of perforators between the different subjects. To give a better intuitive idea, the dots are divided into three groups. The large (red) dots indicate that these perforators have a 'higher' temperature compared to the others (see also figure 4). This fact also implies that they become larger during the rewarming and visually they usually appear to have approximately twice the diameter of the medium dots (yellow). It is probably for these reasons that the red dots are 'seen' first during the rewarming. The yellow dots have a lower temperature and diameter than the red and they appear later in the rewarming. The 'low' temperature dots are smallest (cyan). Some cyan dots may appear at the same time as the yellow, but their size remains small and their borders are less well defined. In general, detection is more difficult on static images and a better procedure is to observe the images in real time or on a recorded 'movie'. In this study, the images were viewed independently by three persons (two radiologists and a physicist). It would be helpful to develop a software programme allowing an automated detection of the perforator vessels; however, this was beyond the scope of the present study.

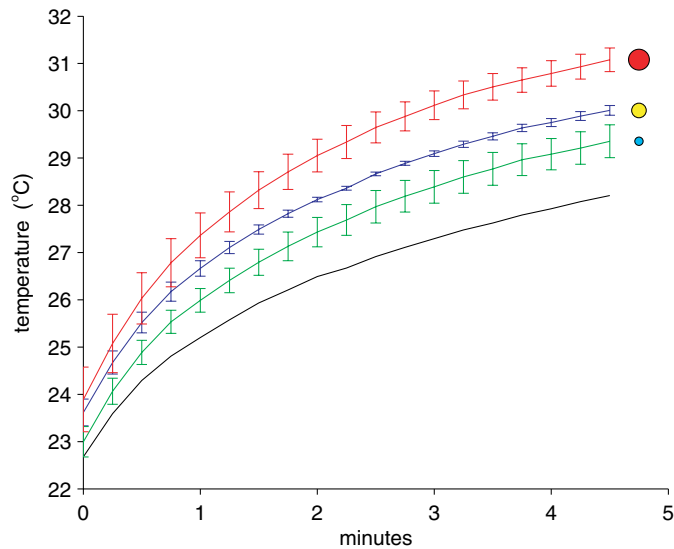


Figure 4. Mean temperature as a function of time for the three groups of perforator vessels appearing in figure 3 (bottom, right). The black line represents the recovery of a typical background region. The vertical bars represent the standard deviation.

Table 1. Number of perforator vessels measured on the region of interest on the *vastus lateralis* muscle for each subject as a function of the intensity appearing in the thermographic images (low, medium and high intensities correspond to cyan, yellow and red colours in figure 1).

Subject no	Low intensity	Medium intensity	High intensity	Total
1	5	7	2	14
2	3	5	1	9
3	3	3	2	8
4	12	17	4	33
5	9	6	2	17
6	8	5	5	18

To better show the temperature behaviour, figure 4 indicates the time-dependent temperature kinetics in the three different groups for a typical subject (corresponding to figure 1). It must be noted that the present classification does not allow one to discriminate a superficial small vessel from a deep larger vessel. Table 1 summarizes the number of perforators of each type observed for each subject.

4. Discussion

In the present work it appears that IR cameras equipped with QWIP detectors are suitable instruments for the noninvasive detection of the anatomical position of perforator vessels. The present results show that the perforator vessels can be easily detected with the method proposed by Itoh and Arai (1995), and due to the sensitivity of the camera it was also possible to observe vessels that only gave a weak infrared signal (e.g. cyan dots in figure 3). These smaller underlying vessels are usually not utilized in plastic reconstructive surgery because only the

large perforators (red dots) may reasonably act as the pedicle of the resected flap and represent real perforators (a large vessel in fact increases the probability of the tissue survival). However, the presence of these smaller vessels may influence NIRS or LDF measurement because both techniques are very sensitive to venule, capillary and arteriole distribution/density changes (Mancini *et al* 1994). If one of the optodes is placed over a perforator vessel, this can obviously produce abrupt changes in the NIRS or LDF signal. Indeed, the accuracy of NIRS measurements has also been shown to vary with the size of larger vessels in the measurement region (Firbank *et al* 1997). The same effect may be observed if the vessel is part of the optodes field of view and thus results from NIRS imaging devices may also depend on perforator distribution. Figure 3 clearly shows that the vessel distribution (coloured dots) varies among subjects and thus it is very difficult to give general advice on how to bypass the problem. The present perforator distribution on the (black) region of interest is compatible with direct surgical observations where only the large septocutaneous or musculocutaneous perforators were obviously considered (Kuo *et al* 2002) and previous studies on cadaver (Taylor *et al* 1994). For simplicity, in figure 3 the perforators are represented as dots. However, they may actually have 'non-circular' shape (figure 2) and they may be more or less close to each other. As explained by Chijiwa *et al* (2000) this is due to the presence of different types of perforator vessels, i.e.: type (I) run to the skin surface; type (II) are linked at the base and run to the skin surface; type (III) run straight to the dermal surface and disperse parallel to the skin. These different morphological structures also contribute to the inhomogeneity of the tissue.

It is evident, from the present work, that the vascular network might explain many of the spatial differences observed in NIRS or LDF measurements of skeletal muscle blood saturation levels (Miura *et al* 2001, Miura *et al* 2003), oxygen consumption (Quaresima *et al* 2001), blood velocity (Binzoni *et al* 2003), etc because these techniques depend on the homogeneity of the vascular tree. Thus if one wants to compare the metabolic activities of two different muscle regions it is mandatory to ensure that the main vascular structure is similar and comparable and that the optodes are not placed on vascular 'singularities' such as the perforator vessels. If these precautions are not taken, metabolic parameters may be easily mixed with tissue perfusion-dependent parameters and lead to wrong conclusions concerning the physiological interpretation of the observed phenomena.

We conclude that accurate comparison of the metabolic activity of two different muscle regions is not possible without reliable maps of vascular 'singularities' such as the perforator vessels and that a QWIP-based imaging system is one method to obtain these data. Furthermore, QWIP-based passive infrared imaging appears to be an efficient, effective, non-invasive method of mapping perforator vessels and may be an important surgical planning tool for reconstructive surgery. A further study is now under way to verify this.

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