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Review

The use of near-infrared spectroscopy in understanding skeletal muscle physiology: recent developments

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This article provides a snapshot of muscle near-infrared spectroscopy (NIRS) at the end of 2010 summarizing the recent literature, offering the present status and perspectives of the NIRS instrumentation and methods, describing the main NIRS studies on skeletal muscle physiology, posing open questions and outlining future directions. So far, different NIRS techniques (e.g. continuous-wave (CW) and spatially, time- and frequency-resolved spectroscopy) have been used for measuring muscle oxygenation during exercise. In the last four years, approximately 160 muscle NIRS articles have been published on different physiological aspects (primarily muscle oxygenation and haemodynamics) of several upper- and lower-limb muscle groups investigated by using mainly two-channel CW and spatially resolved spectroscopy commercial instruments. Unfortunately, in only 15 of these studies were the advantages of using multi-channel instruments exploited. There are still several open questions in the application of NIRS in muscle studies: (i) whether NIRS can be used in subjects with a large fat layer; (ii) the contribution of myoglobin desaturation to the NIRS signal during exercise; (iii) the effect of scattering changes during exercise; and (iv) the effect of changes in skin perfusion, particularly during prolonged exercise. Recommendations for instrumentation advancements and future muscle NIRS studies are provided.

Keywords: skeletal muscle; near-infrared spectroscopy; near-infrared imaging; muscle oxygenation; muscle oxy-haemoglobin saturation; muscle metabolism

1. Introduction

Since the end of the 1980s, near-infrared spectroscopy (NIRS) has been used to investigate local muscle oxidative metabolism at rest and during different exercise modalities. The unique advantage of using NIRS is that, when proper care is

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taken to minimize movement artefacts, it can yield acceptable signal-to-noise ratios during exercise. Ferrari *et al.* [1] reviewed the first decade of NIRS muscle studies at the dedicated 1996 Royal Society Discussion Meeting. In the following years, several other reviews covered different aspects of muscle NIRS research such as sports science and clinical medicine [2,3]. Hamaoka *et al.* [4] reviewed the methodological issues of NIRS and near-infrared (NIR) imaging for monitoring muscle oxygenation and haemodynamics in healthy and diseased humans. The 234 references quoted in that review article witness not only the evolution of the NIRS/NIR imaging techniques, but also their significant applications in exercise physiology and clinical medicine.

A search on the databases PubMed, Scopus and Web of Science was performed using the keywords: 'near-infrared', 'near-infrared oximetry', 'muscle oxygenation' and 'optical imaging'. From 2007 up to the end of 2010, approximately 160 articles related to muscle NIRS studies (excluding the clinical related studies) were published on different aspects of muscle physiology (primarily muscle oxygenation and haemoglobin (Hb) volume) of several upper and lower limb muscle groups investigated by using mainly one-/two-channel commercial NIRS instruments. Only 15 of these studies exploited the advantages linked to the use of multi-channel instruments.

Considering that the number of laboratories and articles related to the application of NIRS in muscle studies is increasing, most muscle physiology researchers would agree with the assertion that muscle NIRS research is progressing. The technology and methodology of NIRS instruments have shown advances in robustness and sophistication. This article is an attempt to provide a snapshot of muscle NIRS at the end of 2010 summarizing the recent literature, offering the present status and perspectives of the NIRS instrumentation and methods, the main fields of applications for studying skeletal muscle physiology, the open questions and future directions.

2. Present status and perspectives of the near-infrared spectroscopy muscle instrumentation and methods

(*a*) *Commercial near-infrared spectroscopy systems used in muscle studies*

The different types of NIRS instruments with the related key features, advantages and disadvantages have been previously reviewed in detail [5]. Briefly, three different NIRS techniques are used, each based on a specific illumination type: (i) the continuous-wave (CW) modality, based on constant illumination of the tissue, simply measures the attenuation of light through the tissues; (ii) frequency-domain (FD) instruments, illuminating the tissues with intensity-modulated light, measure both the attenuation and the phase shift of the emerging light; and (iii) the time-domain (TD) technique, illuminating the tissues with short pulses of light, detects the shape of the pulse after propagation through the tissues. The quantitation of the NIRS measurable parameters depends on the adopted NIRS technology. Different NIRS methods using the CW modality have been developed to measure the oxy-Hb (O_2Hb) saturation of the muscle (SmO_2, %), one being the spatially resolved spectroscopy (SRS) method, which is the most widely used oximetry approach. The SmO_2 measurement ensures an accurate quantitation of the oxygenation changes

occurring at the muscle level. SmO_2 reflects the dynamic balance between O_2 supply and O_2 consumption in the investigated muscle volume. CW-based systems offer the advantages of low cost and easy transportability. In ascending order, CW-, FD- and TD-based instruments require increased cost and technological complexity. On the other hand, only FD and TD techniques offer the absolute characterization of the tissue optical properties (absorption and reduced-scattering coefficients), from which it is possible to retrieve absolute concentration values of O_2Hb and deoxy-Hb (HHb), and a derived parameter total-Hb (tHb = O_2Hb + HHb). Moreover, a scaled absolute value for tHb can be obtained by the SRS method. NIRS methodology is characterized by a relatively high temporal resolution (sampling rate up to 100 Hz with CW- and FD-based instruments, and around 5 Hz with TD-based instruments) enabling the measurement of the time course of changes in SmO_2 during brief (2–4 s) leg press exercise [6] or even during a single pedal cycle of a cycling exercise [7].

Table 1 reports the main current commercial NIRS instruments used in muscle studies. The details of each system can be found in the relevant company's website. Most of the instruments are represented by two-channel brain oximeters used also in muscle studies. These oximeters are the most commonly used instruments for muscle studies because they are of relatively low/moderate cost and transportable. The NIRS instrumentation can be miniaturized and even made wireless. Unfortunately, no standardization is yet available for NIRS instrumentation. In particular: (i) the operating range (i.e. the interval within which the instrument works reliably) of the oximeters should be recognized and indicated and (ii) comparison between oximeters at rest and during dynamic exercise should be performed.

Recent advances in NIRS technology have included the introduction of NIR multi-channel systems (which use arrays of multiple NIR sources and detectors) that, by simultaneously collecting data from multiple muscle regions, avoid variations caused by position-dependent differences in muscle oxygenation (a problem inherent with single-location measurements). Multi-channel NIRS systems provide only topographic NIR images (i.e. two-dimensional images) and, in this article, they are referred to as 'imagers'. Typical depth sensitivity of the CW-based imagers is approximately 1.5 cm and the spatial resolution is limited to approximately 1 cm. TD- and FD-based imagers are the most powerful tools to investigate the spatial and temporal profiles of the absolute changes in SmO_2 and tHb during exercise; however, the signal-to-noise ratio of measurements is low. Although prototypes of muscle NIR imagers have been available since the end of the 1990s, NIR imaging has been rarely used in muscle studies probably owing to its relatively high cost and complexity compared with one-two-channel oximeters.

(*b*) *New methods and signal interpretability*

The muscle parameters measurable directly and indirectly by NIRS and NIR imaging have been reviewed by Wolf *et al.* [5]. Muscle NIRS parameter measurements are repeatable and reproducible during elbow flexor exercise [8] and cycling exercise [9].

Table 1. Main current commercial near-infrared spectroscopy systems used in muscle studies. CW, continuous-wave spectroscopy; FD, frequency-domain spectroscopy; TD, time-domain spectroscopy; u.r., upon request.

instrument	technique	no. of channels	company	website
photometers				
BOM-L1 TR[a]	single-distance CW	1	Omegawave, Japan	www.omegawave.co.jp
PocketNIRS Duo[a,b]	single-distance CW	2	Hamamatsu, Japan	www.hamamatsu.com
oximeters				
Astem Hb-12c[a–c]	multi-distance CW	2	Astem, Japan	www.astem-jp.com
InSpectra 650[d,e]	multi-distance CW	1	Hutchinson, USA	www.htbiomeasurement.com
INVOS 5100C[d,f]	multi-distance CW	4	Somanetics, USA	www.somanetics.com
NIMO[c]	multi-distance CW	1–4	Nirox, Italy	www.nimoworld.com
NIRO-200NX[f]	multi-distance CW	2	Hamamatsu, Japan	www.hamamatsu.com
OXYMON-II A[f]	multi-distance CW	2	Artinis, The Netherlands	www.artinis.com
OxiplexTS	multi-distance FD	2	ISS, USA	www.iss.com
PortaMon[b,g]	multi-distance CW	1	Artinis, The Netherlands	www.artinis.com
TRS-20[a,f]	TD	2	Hamamatsu, Japan	www.hamamatsu.com
multi-channel systems[h]				
Dynot	CW	u.r.	NIRx, USA	www.nirx.net
FOIRE-3000[d]	CW	52	Shimadzu, Japan	www.med.shimadzu.co.jp
Imagent	FD	up to 128	ISS, USA	www.iss.com
NIRO-200	CW	10	Hamamatsu, Japan	www.hamamatsu.com
NIRS2 CE, CW6	CW	up to 176	TechEn, USA	www.nirsoptix.com
OXYMON MkIII	CW	up to 96	Artinis, The Netherlands	www.artinis.com

[a]Commercially available only in Japan.
[b]Wireless system.
[c]Oximeter with fat-layer compensation in real time.
[d]US Food and Drug Administration's approval.
[e]Only for the thenar muscle.
[f]Brain oximeter also used in muscle studies.
[g]An accelerometer is available on request.
[h]Multi-channel systems have been developed for cortical activation studies and scarcely used on muscle.

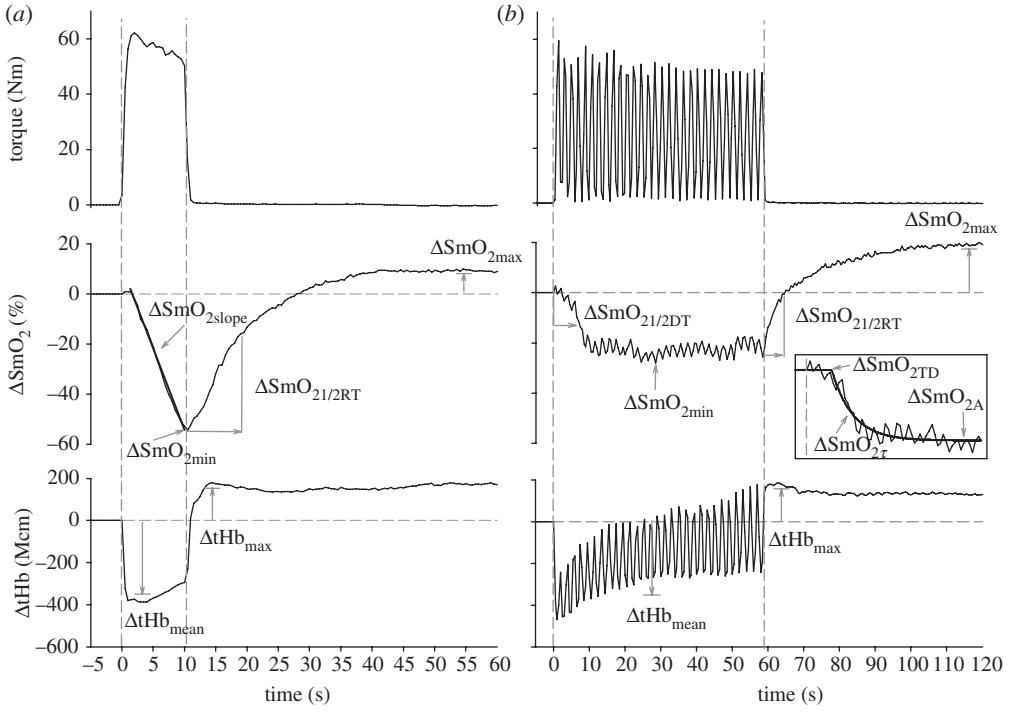

Figure 1. Typical changes in elbow flexor torque and biceps brachii muscle oxyhaemoglobin saturation (ΔSmO_2) and total haemoglobin volume (ΔtHb) during a 10 s sustained (*a*) and 30 repeated (*b*) isometric exercise task at 100% maximal voluntary contraction. The vertical dotted lines delimit the duration of the exercise. ΔSmO_{2slope}, ΔSmO_2 desaturation slope; $\Delta SmO_{2\,min}$, minimum ΔSmO_2 amplitude; $\Delta SmO_{2\,max}$, maximum ΔSmO_2 amplitude; $\Delta SmO_{21/2RT}$, ΔSmO_2 half resaturation time; $\Delta SmO_{21/2DT}$, ΔSmO_2 half desaturation time; ΔtHb_{mean}, ΔtHb mean decrease; ΔtHb_{max}, ΔtHb maximum increase. Inset (*b*): SmO_2 kinetic analysis using nonlinear regression analysis. SmO_{2TD}, initial time delay; $SmO_2\tau$, exponential time constant; ΔSmO_{2A}, ΔSmO_2 desaturation amplitude.

An example of the typical changes in biceps brachii SmO_2 and tHb (measured by a two-channel oximeter) during a 10 s sustained and 30 repeated isometric exercise of the elbow flexors at maximal voluntary contraction is shown in figure 1. During the sustained contraction (figure 1*a*), ΔSmO_2 desaturation slope (ΔSmO_{2slope}) is the negative slope of the least-squared regression line of ΔSmO_2 during the contraction phase; a higher ΔSmO_{2slope} would represent a greater muscle O_2 demand, and consequently a greater energy consumption [8,10]. ΔSmO_2 minimum amplitude ($\Delta SmO_{2\,min}$) is the difference between the minimum SmO_2 value reached during the contraction phase and SmO_2 baseline; lower $\Delta SmO_{2\,min}$ values represent greater O_2 demand relative to O_2 supply [6,10]. ΔSmO_2 maximum amplitude ($\Delta SmO_{2\,max}$) is the difference between the maximum ΔSmO_2 value reached during the relaxation phase and SmO_2 baseline; higher ΔSmO_{2max} values indicate increased O_2 supply relative to O_2 demand [6]. ΔSmO_2 half-recovery time ($\Delta SmO_{21/2RT}$) is the time to reach 50 per cent of the difference between ΔSmO_2 at the end of the contraction phase and ΔSmO_{2max} in

the recovery period; a faster $\Delta SmO_{21/2RT}$ is related to muscle oxidative capacity [8]. Mean decrease in ΔtHb (ΔtHb_{mean}) is the difference between the average ΔtHb value during the contraction phase and tHb baseline; greater decreases and relatively stable ΔtHb_{mean} values during the contraction phase would indicate blood flow/O_2 supply occlusion owing to increased intramuscular pressure of the contraction [11]. Maximum increase in ΔtHb (ΔtHb_{max}) is the difference between the maximum ΔtHb value reached during the relaxation phase and tHb baseline; higher ΔtHb_{max} values would indicate a greater increase in blood volume/flow [6].

During dynamic exercise (figure 1*b*), $\Delta SmO_{2\,min}$ is the difference between the minimum $\Delta SmO_{2\,min}$ value reached during the 30 contractions and SmO_2 baseline; a given $\Delta SmO_{2\,min}$ value represents the dynamic balance of O_2 supply by the microcirculation and O_2 consumption by the mitochondria [8]. ΔSmO_2 desaturation time ($\Delta SmO_{21/2DT}$) is the time difference between contraction onset until ΔSmO_2 reaches 50 per cent of the difference value between SmO_2 baseline and $\Delta SmO_{2\,min}$; a longer duration $\Delta SmO_{21/2DT}$ for a similar $\Delta SmO_{2\,min}$ would represent a slower desaturation rate, indicating that O_2 demand is better matched by O_2 supply. ΔtHb_{mean} is the difference between the average of the minimum ΔtHb amplitude reached during the 30 contractions and tHb baseline; a given ΔtHb_{mean} value would indicate the level of blood volume or blood flow/O_2 supply over the exercise duration [11].

The kinetics of muscle oxygenation can be modelled by nonlinear regression using least-squares techniques [12], and the kinetics of SmO_2 is represented by the following formula:

$$SmO_2(t) = SmO_{2base} - SmO_{2A}(1 - e^{-(t-SmO_{2TD})/SmO_2\tau}),$$

where SmO_{2base} represents the resting SmO_2 baseline value. The curve fit for the first 30 s of exercise is shown in the inset of figure 1. After the onset of exercise (represented by the first dashed line), an initial time delay (SmO_{2TD}), followed by a rapid desaturation in SmO_2, is described with an exponential time constant ($SmO_2\tau$) representing the time to reach 63 per cent of the desaturation response. SmO_2 desaturation amplitude (SmO_{2A}) is the difference between the SmO_{2base} and the nadir of SmO_2. The SmO_2 mean response time ($SmO_{2MRT} = SmO_{2TD} + SmO_2\tau$) can be calculated to provide a description of the overall time course for muscle desaturation during the rest–exercise transition. A faster SmO_{2MRT} would indicate that O_2 consumption is not sufficiently being matched by O_2 supply, and conversely, a slower SmO_{2MRT} would suggest that O_2 consumption is sufficiently being matched by O_2 supply [12].

Many studies have evaluated the kinetics of muscle oxygenation using CW–SRS-based oximeters and have drawn their physiological conclusions only on the basis of the interpretation of the changes in HHb. Several arguments have been raised to support [13] or refute [14] this choice, because these oximeters provide SmO_2 values that are independent of the pathlength of the NIR photons in the muscle tissue and, unlike HHb, are not so sensitive to the optical coupling and to the presence of superficial tissue layers. Taking into account the advantages offered in using CW–SRS-based oximeters, the physiological conclusions should be drawn essentially on the basis of SmO_2 results, and reporting tHb data.

Among the most recent methods, Leung *et al.* [15] determined SmO_2 during cycling exercise measuring the rhythmical changes of the NIR signal or ‘cyclic

SmO_2'. Binzoni *et al.* [7] further developed Leung's method by using the 'cyclic' signal embedded in the NIR signal to derive instantaneous SmO_2 and muscle O_2 consumption.

(*c*) *Advanced near-infrared imaging technologies and multi-modal spectroscopy/imaging*

Among the advanced commercial NIR technologies used for muscle studies in the last 4 years, it is significant to mention the following three approaches: (i) CW–SRS imaging, (ii) TD imaging, and (iii) wireless CW imaging. Kek *et al.* [16] developed a portable muscle NIR imager, based on CW–SRS, which is capable of measuring SmO_2 over 32 measurement points (with a fat-layer correction algorithm) during quadriceps dynamic exercise. TD muscle imaging instruments have the advantage of improving the depth sensitivity by exploiting the temporal information of photon migration through tissues (i.e. early photons primarily representing superficial layers and late photons representing deeper muscle layers). The Politecnico of Milan (Italy) developed a two-wavelength TD imager with a sampling time of 6 Hz and allowing up to 32 measurement points [17].

NIR imaging systems use cables to connect the sensor that is attached to the subject to the acquisition electronics. These cables are disturbing and can cause motion artefacts by dislocating the sensor if care is not taken to secure the cables properly. For these reasons, a lightweight wireless NIR imager was developed [18].

In the last 20 years, muscle NIRS has been employed also in combination with a large variety of other non-invasive and invasive methodologies for evaluating pathophysiological changes in peripheral muscle (for a review see Hamaoka *et al.* [4]). A recent study demonstrated the usefulness of the combination of NIRS with nuclear magnetic resonance (NMR) techniques for obtaining robust information regarding muscle oxidative metabolism and haemodynamics [19].

3. Main fields of near-infrared spectroscopy applications for studying skeletal muscle physiology

So far, only about 20 out of the approximately 600 skeletal muscles have been investigated by NIRS. Specifically, lower limb muscles (i.e. biceps femoris, gastrocnemius, rectus femoris, tibialis anterior, vastus lateralis, vastus medialis) were studied during diverse conditions (cycling, upon electrical stimulation, during knee extension exercise, leg press exercise, plantar flexion exercise, running, squatting, Wingate test), and upper limb muscles (i.e. biceps brachii, brachioradialis, deltoid, forearm flexors, triceps brachii) during diverse conditions (arm abduction exercise, bench press exercise, cycling, elbow flexion exercise, upon electrical stimulation, handgrip, rowing, Wingate test). The trunk muscles (i.e. erector spinae, intercostal, multifidus, paravertebral, serratus anterior) were also investigated during cycling, back extension and bending forward.

So far, NIRS has been applied for studying exercise-induced muscle damage [11,20], ergonomics/biomechanics [21], heterogeneity of muscle O_2 supply/demand [12,22], muscle activation [11,23], priming exercise [19,24], respiratory muscle blood flow/fatigue [25,26], the role of the brain in muscle fatigue [27,28], the time course of oxidative metabolism [6,29] and the effect of

exercise training [30]. Owing to the restriction of the allocated space, this review article neglects an in-depth discussion of the 160 studies published in the last four years.

It is important to mention that among the muscle NIRS articles published in the last four years only 15 were performed by using NIR imagers. It is envisioned that more studies in the future will use the benefits of NIR imagers to study regional differences in muscle O_2 supply–demand responses to exercise. The effects of different motor tasks [31] and the effects of exercise/muscle fatigue [32] on both muscle and cortical oxygenation changes have been previously reviewed.

4. Open questions

Some of the most significant open questions in order of importance are: (i) the possibility to use NIRS in subjects with a large fat layer; (ii) the contribution of myoglobin (Mb) desaturation to the NIRS signal during exercise; (iii) the effect of scattering changes during exercise on CW–NIRS muscle measurements; (iv) the effect of changes in skin perfusion on CW–NIRS muscle measurements, particularly during prolonged exercise; and (v) the possibility to measure muscle/interstitial pH.

It is well known that the relatively high attenuation of NIR light in muscle measurements is owing to: (i) the two main chromophores—Hb and Mb; (ii) light scattering; and (iii) other molecules (mainly skin melanin, water, lipids of the adipose tissue, intra-muscle lipids and cytochrome c oxidase). The influence of adipose tissue thickness (ATT) on light propagation in leg muscles has been examined by several researchers. Muscle NIRS testing has been limited to slim subjects (preferably men because they have a smaller ATT with respect to women). Patients with diabetes, paraplegia or chronic fatigue syndrome, who have a tendency to be obese, cannot be investigated by NIRS if ATT values are high. At least three methods have been developed for fat-layer correction. An algorithm capable of correcting for the influence of ATT ranging from 0 to 15 mm has been proposed [33] and it has been included in some commercial NIRS systems (table 1). Another ATT correction algorithm and an optical method for ATT monitoring have been developed [34,35]. The optical lipid signal is a good predictor for ATT less than 16 mm. The possibility of measuring ATT directly might be an efficient alternative to the measurement of ATT by ultrasound. Another method for measuring SmO_2 has been recently reported [36]. This method uses broadband CW–NIRS after the removal of spectral interference owing to skin, water, ATT and scattering. Unfortunately, none of these methods have been so far used by the most widespread commercial NIRS system manufacturers (table 1), which strongly limits the clinical applicability of muscle NIRS in patients with high ATT.

There is a considerable discussion about the contribution of Hb and Mb to the *in vivo* NIR signal from skeletal muscle. It is difficult to differentiate Hb and Mb spectra because they are very similar in the NIR range. Marcinek *et al.* [37], using wavelength shift analysis, showed that Hb accounts for approximately 20 per cent of the optical signal in human resting first dorsal interosseous muscle. However, Hb/Mb ratio in the muscle at rest is unknown, and there are few data available on the relative kinetics of Mb and Hb desaturation during different

exercise modalities. In an animal study, Mb desaturation of rat gastrocnemious muscle was investigated by NIRS during Hb-free medium perfusion [38]. At the onset of contraction, Mb desaturated rapidly and declined progressively with work intensity. Proton (^{1}H) NMR measures muscle deoxy-Mb signal allowing the assessment of intracellular O_2 availability at rest and during exercise with a time resolution of about 8 s. In a human study, Lanza *et al.* [39] found that, at rest, the tibialis anterior intramuscular O_2 stores (measured by the appearance of ^{1}H NMR deoxy-Mb signal during cuff occlusion) began to decrease only after 1 min, and that maximal Mb desaturation was achieved after about 6.5 min. Conversely, at rest, the intramuscular O_2 stores (measured by NIRS during cuff occlusion) in different muscle groups began to decrease a few seconds after the beginning of the occlusion and maximal desaturation was achieved 4–5 min later. During high-intensity exercise, Mb typically desaturates to only 50 per cent of the level attained during cuff occlusion, and muscle oxygenation, as measured by CW–NIRS, typically desaturates to about 90 per cent of the level attained during the cuff occlusion. Overall, these data would suggest that the contribution of Mb desaturation to the NIR signal during dynamic exercise is estimated to be roughly less than 20 per cent. Combined ^{1}H NMR and NIRS studies could clarify not only the issue of the contribution of Mb to the NIRS signal, but also the relative kinetics of Mb and Hb desaturation during exercise with different workloads.

In principle, exercise could alter the muscle tissue optical properties by causing a change in the scattering of NIR photons. The change in scattering at different wavelengths and the relative pathlength changes that occur during different exercise modes in the human quadriceps and calf muscles have been studied by TD–NIRS techniques [40,41]. It was observed that scattering coefficients and pathlength in the muscle, at different wavelengths, decreased by less than 10 per cent during the different exercise modalities. Therefore, it could be expected that scattering and pathlength changes, occurring during exercise, would negligibly contribute to the calculation of changes in O_2Hb, HHb and tHb measured by CW–NIRS instruments.

Several controversial studies have been published on the effect of skin blood flow changes on muscle NIRS measurements. A recent study [42] provided reassurance that changes in SmO_2 during exercise predominantly reflect muscle oxygenation, even during conditions where skin and muscle blood flow were elevated concomitantly (e.g. during prolonged, dynamic exercise).

Soller *et al.* [43] and previous studies showed that broadband CW–NIRS can be used to non-invasively and continuously measure SmO_2 (based on Hb spectral features), and indirectly muscle/interstitial fluid pH. Partial least-squares regression was used to develop the multi-subject calibration equation, which correlates NIR spectra to muscle/interstitial fluid pH values obtained invasively in the muscle of the calibration subjects. Once developed, the equation can be applied to NIR spectra acquired from independent subjects to predict muscle/interstitial pH. Recent studies demonstrated the feasibility of the muscle pH estimation, the determination of H^+ threshold and the relation between H^+ and classical metabolic thresholds (lactate and gas exchange) during incremental exercise [44].

A summary of the recommendations for properly using NIRS instrumentation in muscle studies is reported in table 2.

Table 2. Recommendations for near-infrared spectroscopy muscle studies. ATT, subcutaneous adipose tissue thickness; CW, continuous-wave spectroscopy; EMG, electromyography; FD, frequency-domain spectroscopy; fMRI, functional MRI; MRI, magnetic resonance imaging; NMR, nuclear magnetic resonance spectroscopy; SRS, spatially resolved spectroscopy; PET, positron emission tomography; TD, time-domain spectroscopy.

topic	current status	recommendations
depth sensitivity	typically approximately 1.5 cm for 3–4 cm source–detector distance. Measurements restricted to superficial muscle(s)	use TD technologies and tomographic approaches for improving depth sensitivity. In the case of multi-distance CW–NIRS, use less than 5 mm and greater than 30 mm for the shortest and longest source–detector distance, respectively
investigated muscle volume and measurement points	oxygenation of large muscle groups like the quadriceps is investigated by using only one or two measurement points	use multi-channel systems for investigating the spatial profile of muscle/muscle groups oxygenation
optode positioning	often not accurately reported	describe in detail the location, eventually guided by ultrasound scanner
optode–skin coupling/sliding; optode sliding owing to sweat (especially in hairy skin) and/or mechanical factors	often not verified during and after the study or not mentioned	ensure an adequate stable contact between the optodes and the skin throughout the acquisition session. Minimize the sliding by bandage (avoiding venous occlusion), and use NIR transparent double-sided adhesive tape. Monitor the pressure of the optode on the skin
adipose tissue thickness	often not reported	measure ATT using skinfold callipers, ultrasound scanner, MRI or optical lipid signal. Eventually perform studies only on subjects with homogeneous ATT. Use algorithms for ATT correction
skin blood flow changes over the exercising muscle	usually not measured	measure skin blood flow (e.g. by laser Doppler) and/or skin temperature close to the optode in prolonged exercise
muscle shape changes during exercise	usually not mentioned	try to keep the limb movements in the same planes in order to minimize artefacts. Artefacts should be identified and corrected/eliminated in the NIRS data analysis
exercise and experimental set-up description	often inaccurate	describe in detail the protocol for the reproducibility/repeatability of the measurements

(*Continued*.)

Table 2. (*Continued.*)

topic	current status	recommendations
kinematic motor performance	motor performance not always monitored and controlled	monitor motor performance by three-dimensional kinematic analysis in particular for open field exercise
absolute quantification value of NIRS measures	SRS–CW-based systems provide only SmO_2 (%) quantification	use TD- and FD-based instrumentations for improving sensitivity and quantitation of NIRS parameters
data analysis	analysis of SmO_2 (%) and concentration changes in O_2Hb, HHb and tHb. Often only the HHb kinetics and amplitude are analysed and reported	analyse and report all measurable parameters, i.e. O_2Hb, HHb, tHb, SmO_2
standardization	no standardization is available for NIRS instrumentation/signal processing/data analysis	regulatory authorities or network of research laboratories should provide 'guidelines'
multi-modal studies	very few studies	integrate NIRS with MRI, fMRI, NMR, PET, EMG, microdialysis, Doppler blood flow measurements

5. Future directions

The most exciting prospect of muscle NIRS studies for the next 20 years is the full understanding of skeletal muscle biochemistry/physiology/pathology for improving human healthcare, athletic performance and rehabilitation monitoring. The major challenge to achieving this understanding might be the availability of a low-cost, easy-use optical wearable/wireless non-contact NIR imager for obtaining four-dimensional SmO_2 and haemodynamic (blood flow and tHb) measurements of human skeletal muscle, especially during dynamic exercise. This ideal 'NIR imager' should be suitable for any application (including general health, clinical and athletic settings), and might be an addition to the current heart rate monitoring and lactate measurements during training in the field and health centre-like environments. This ideal 'NIR imager' should be combined/integrated with other imaging and electrophysiological modalities for enhancing the understanding of specific muscle mechanisms in pathophysiological conditions.

Considering the rapid development of related technologies, it is very difficult to predict the potential advancements of muscle NIRS and NIR imaging. The quantitative measurement of deep forearm oxygenation and tHb by a non-contact oximeter prototype was proposed by Niwayama *et al.* [45].

The current typical depth sensitivity of most CW-based imagers is approximately 1.5 cm. Therefore, a tomographic approach might provide three-dimensional SmO_2 and haemodynamic measurements. Blood flow of

the superficial muscles might be continuously measured by diffusing-wave spectroscopy, a new rapidly progressing technique discussed in an accompanying symposium paper. Although three-dimensional NIR imaging of the human forearm, based on TD techniques, was proposed by Hillman *et al.* [46] almost 10 years ago, no further progress has been made to develop the technique.

In conclusion, it is foreseeable that the availability of advanced NIR imagers would help to refine the understanding of skeletal muscle oxygenation in different pathophysiological conditions.

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