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Review

The use of muscle near-infrared spectroscopy in sport, health and medical sciences: recent developments

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Near-infrared spectroscopy (NIRS) has been shown to be one of the tools that can measure oxygenation in muscle and other tissues *in vivo*. This review paper highlights the progress, specifically in this decade, that has been made for evaluating skeletal muscle oxygenation and oxidative energy metabolism in sport, health and clinical sciences. Development of NIRS technologies has focused on improving quantification of the signal using multiple wavelengths to solve for absorption and scattering coefficients, multiple pathlengths to correct for the influence of superficial skin and fat, and time-resolved and phase-modulated light sources to determine optical pathlengths. In addition, advances in optical imaging with multiple source and detector pairs as well as portability using small wireless detectors have expanded the usefulness of the devices. NIRS measurements have provided information on oxidative metabolism in various athletes during localized exercise and whole-body exercise, as well as training-induced adaptations. Furthermore, NIRS technology has been used in the study of a number of chronic health conditions. Future developments of NIRS technology will include enhancing signal quantification. In addition, advances in NIRS imaging and portability promise to transform how measurements of oxygen utilization are obtained in the future.

Keywords: muscle; near-infrared imaging; oximetry; muscle oxygenation; muscle oxidative metabolism; exercise

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1. Introduction

This paper primarily reviews the most recent (from 1996 to 2010) muscle near-infrared spectroscopy (NIRS) studies for evaluating skeletal muscle oxygenation and oxidative metabolism and discusses some of the most exciting applications of NIRS technology for the next two decades, specifically in sport, health and medical sciences. A brief description of the biochemistry and physiology of muscle oxidative metabolism is presented, and then the basic principle of the NIRS measurement, examples of muscle NIRS measurements, the limitations of the methodologies and the prospect of NIRS applications to this area are demonstrated to better understand the monitoring of muscle oxidative functions. Detailed review articles recently published can be found elsewhere [1–5] regarding the principles, limitations and applications of NIRS in muscle exercise physiology and pathology.

(*a*) *Biochemistry and physiology of muscle oxidative metabolism*

Skeletal muscle possesses two major biochemical processes for synthesizing adenosine triphosphate (ATP), namely oxidative phosphorylation and anaerobic ATP production (phosphocreatine (PCr) and glycolytic pathways). During a low-to-moderate intensity of exercise at which we normally perform in a daily activity, skeletal muscle deeply relies on oxidative metabolism. During exercise, skeletal muscle O_2 consumption (VO_2) can rise 50-fold, with abrupt increases in O_2 delivery (DO_2) of up to 10-fold.

Owing to the strong dependence of skeletal muscle on oxidative metabolism, improvements in the oxidative system of the body create higher performance in an athletic event. On the other hand, impairment of VO_2 and/or DO_2 will limit exercise performance, leading to a functional deterioration.

(*b*) *Practical usefulness of near-infrared spectroscopy*

Invasive methods have been applied to evaluate muscle oxidative metabolism, including peripheral and cardiorespiratory measurements. Peripheral measurements include tissue O_2 microelectrodes, myoglobin O_2 saturation by spectrophotometric analysis [6] and nicotinamide adenine dinucleotide, reduced (NADH) analysis from exposed tissue surfaces [7]. In contrast, magnetic resonance spectroscopy (MRS) has been developed to measure *in vivo* active forms of high-energy phosphorus metabolites and intramuscular pH [8,9]. Since then, MRS has evolved into the 'gold standard' for non-invasive detection of skeletal muscle bioenergetics. However, MRS is rather expensive and requires careful maintenance for precise monitoring. Positron emission tomography, microdialysis and Doppler ultrasound are other modalities for measuring muscle perfusion and aerobic and anaerobic metabolism. In comparison to these technologies, the strength of NIRS for measuring skeletal muscle oxidative metabolism is its non-invasive and portable nature. NIRS devices can make biochemical measurements at frequent intervals on even frail or vulnerable populations, and can be employed in both laboratory- and field-based studies. The ability to collect data during human locomotion is why NIRS lends itself to the study of exercise and athletic performance.

2. Basic principle for *in vivo* muscle near-infrared spectroscopy

NIR devices use wavelengths in the range of 700–900 nm, as this range has a much better penetration into biological tissue than visible light [10]. NIR single-distance continuous-wave spectroscopy (NIR_{SDCWS}) can only provide the relative values of tissue oxygenation, but is simple and portable enough for widespread use. To calculate the changes in oxygenated haemoglobin (Hb)/myoglobin (Mb), deoxygenated Hb/Mb and total Hb/Mb, the equation of a two- or multiple-wavelength method can be applied according to the modified Beer–Lambert law [11].

The similar absorption spectra of Hb and Mb make it difficult to differentiate the two by optical properties alone, but ^{1}H-MRS using the water suppression method has successfully distinguished between the two molecules [12], at least in the case of their deoxy forms. It has been suggested that NIRS-measured SO_2 values reflect predominantly (at least 80%) HbO_2 saturation during exercise in humans [3]. However, Tran *et al.* [13] reported a greater contribution of the Mb signal than Hb to the overall NIR signals in a study using ^{1}H-MRS. The study found that the position of the deoxy-heme peak at 760 nm is linear with Hb/Mb contributions and that Hb accounts for only 20 per cent of the overall signal in human muscles [14]. The differing conclusions from these studies highlight the need for additional studies to clarify not only the issue of the contribution of Mb to the NIR signal, but also the kinetics and the amount of Mb desaturation during exercise under different conditions.

The pattern of the light path follows a 'banana-shaped' curve in which the penetration depth into the tissue is approximately equal to half the distance between the light source and the detector [15]. Subcutaneous adipose tissue greatly influences the NIR signal intensity from the muscle tissue below [5]. For example, an overlying fat thickness of 5 mm reduces the signal intensity by approximately 20 per cent, with a light source–detector separation of 30–40 mm. The use of shorter separation distances, 15–20 mm, attenuates the signal by 30–60 per cent [16]. To account for the effect of adipose tissue thickness on the muscle NIRS signal, correction algorithms have been developed using a combination of short separation distances (0–15 mm) along with longer separation distances (15–40 mm) [16]. Several currently available NIRS units make use of the multiple source–detector pair approach to obtain signals from skeletal muscle independent of superficial tissue [17,18].

The absolute optical pathlength can be measured by NIRS methodologies, including time-resolved spectroscopy (NIR_{TRS}) and phase modulation spectroscopy (NIR_{PMS}) [19]. Nevertheless, there are limited data available regarding changes in optical pathlength during varying interventions, such as arterial occlusion, muscular contractions and recovery from hyperaemia, although it has been reported that changes in pathlength were less than 10 per cent during and after the end of arterial occlusion and during exercise [20,21]. Recently, NIR diffuse correlation spectroscopy (NIR_{DCS}) and diffuse reflectance spectroscopy (NIR_{DRS}) have been developed for measuring changes in muscle oxygenation and muscle blood flow, and are able to compute muscle VO_2 [17].

Two basic approaches have been developed for the presentation of NIRS signals. One is to present changes in both the absolute and relative changes in light absorption. These measurements have been 'validated' with the use of '*in vitro*

yeast and blood models', showing that the subtracted signals at 760 and 850 nm showed a linear relationship to Hb desaturation [15,22,23]. The second approach has been to develop tissue oxygen saturation algorithms based on normoxic–hypoxic animal models. In the USA, the Food and Drug Administration has approved several NIRS devices for clinical use based on this approach. The direct presentation of values based on light absorption has the advantage of being more applicable to a wider variety of conditions and the complete range of oxygen tensions. The advantage of the clinical calibration approach is the administrative approval of the results, necessary for clinical studies, although it should be noted that the results are only valid over the ranges tested in the calibration experiments.

3. What has been the most important/exciting innovation in the last 20 years?

(*a*) *Various calibrations including the physiological arterial occlusion method and fat adjustment*

Without any calibrations, the *in vivo* NIR_{SDCWS} measurement can provide incorrect values of tissue oxygenation owing to a varying layer thickness of tissue [5]. While other methods of calibrating NIRS signals (multiple pathlength and multiple source–detector pairs) have made progress, it is the use of a 'physiological calibration' that has allowed the most practical comparisons of oxygenation levels between different people and different locations. This physiological calibration method creates functional scaling from zero (after 5–6 min of ischaemia) to 100 per cent oxygenation (peak hyperaemia 30–60 s after ischaemia) [15,24].

The use of a physiological calibration in combination with MRS measurements has allowed NIRS to measure muscle oxygen consumption (figure 1) [24]. Assuming that the *in vivo* P/O_2 ratio is 4.6, the rate of decline of muscle oxygenation (O_2Hb) during ischaemia can be compared with the rate of PCr decline. The obtained value of resting metabolic rate in millimoles PCr (or ATP) per second can be converted to millimoles O_2 per second. NIR_{TRS} has also been used to measure resting muscle oxygen consumption (mVO_2), providing results in absolute units [21]. The transient arterial occlusion method has also been used to measure forearm muscle metabolism during exercise [24–26]. Most previous studies have validated NIRS measurements relative to established invasive methods [2,21,23,27,28]. In contrast, a few studies have demonstrated a dissociation of NIRS oxygenation indicators and venous O_2 saturation [29,30]. These results might be explained by changes in oxygenated blood in non-metabolically active tissue (adipose tissue above the exercising muscle), such that some investigators focus only on the deoxygenated signal (assuming it can come only from metabolically active tissue) [31]. However, other investigators have suggested that monitoring Hb oxygen saturation is a better approach to tracking changes in oxygenation in muscle and brain tissues, as they feel that the deoxygenated Hb signal is not always insensitive to blood volume changes during exercise (deoxygenated Hb changes might represent tissue oxygenation changes only when total Hb is stable) [32]. Thus, which signal to use for analysis from the NIRS device may depend on the experimental conditions related to the particular experiment being performed and the physical characteristics of the study participant.

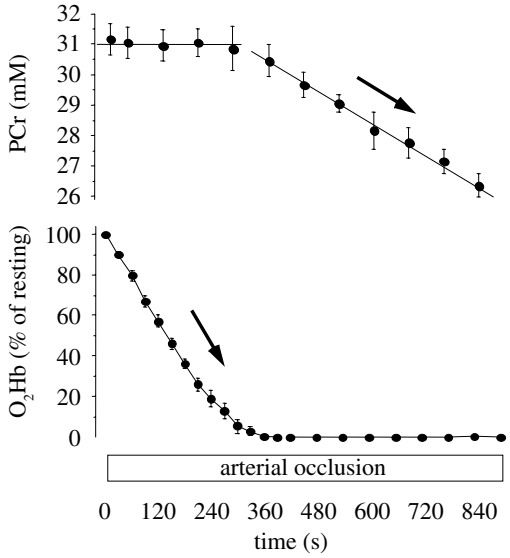

Figure 1. An example of how to calibrate the NIRS signal quantitatively. Changes in phosphocreatine (PCr) and oxygenated haemoglobin and myoglobin (O_2Hb) in the forearm muscle were measured during 15 min of arterial occlusion by MRS and NIRS. The detailed description of the methods is described in a previous study [24]. Copyright © The American Physiological Society. Reproduced with permission of the publisher.

Another major advance in quantification of NIRS signals was to use the onset and recovery kinetics of oxygen saturation to evaluate oxygen use and oxygen delivery [15]. The earlier method of using NIRS signals was to measure changes in deoxy-Hb/Mb signals during ramp cycling exercise to differentiate trained cyclists from physically active subjects [33]. Several studies have used the kinetic approach by measuring the rate of recovery of oxygen saturation with NIRS to evaluate blood flow in the calf muscles of people with peripheral arterial disease [34,35]. The rate of reoxygenation after brief high-intensity maximum voluntary contraction (MVC) exercise is among several indicators for evaluating muscle oxidative capacity [36]. These studies have reported a good agreement between faster PCr recovery kinetics and faster oxygenation kinetics measured with NIRS [37]. Previous studies that examined the association between muscle oxygenation and mVO_2 using NIRS suggest that the initial rate of muscle deoxygenation during transient arterial occlusion is a direct measure of mVO_2 and that the muscle oxygenation level itself is a reflection of mVO_2 [4]. In a study, a new method was proposed to non-invasively approximate muscle capillary blood flow kinetics from the kinetics of the primary component of pulmonary O_2 uptake and deoxy-Hb/Mb in humans during exercise [18].

(*b*) *Multi-channel device*

Early models of NIRS devices used single-distance continuous-wave light source–detector pairs [15]. Later, multi-channel or imaging devices were built to try to measure wider areas of the limb [38,39]. Multiple source–detector pairs have

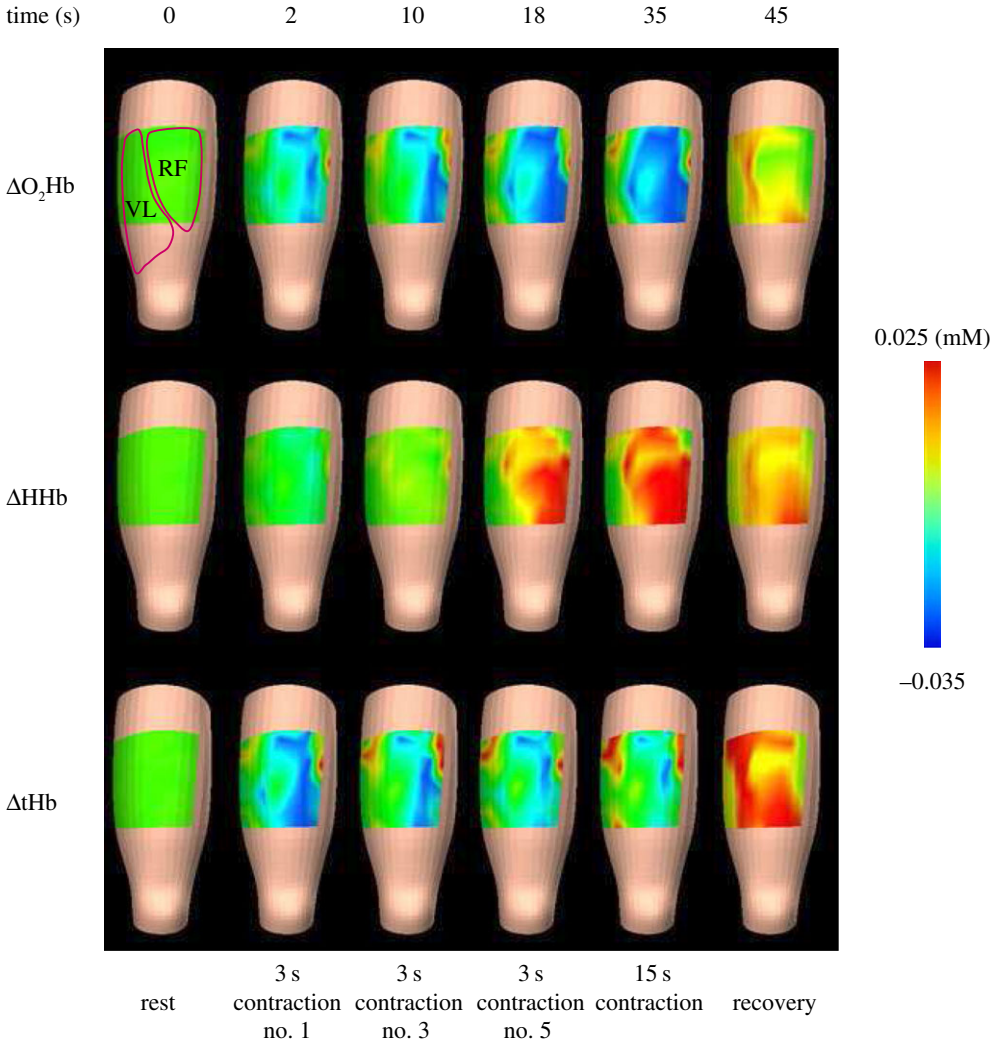

Figure 2. Multi-channel near-infrared (NIR) images during and after muscle contractions. The exercise consisted of repeated 3 s contractions at 50% of MVC with 1 s intervals. The detailed explanation for the device is described in a previous paper [16]. O_2Hb, oxygenated haemoglobin and myoglobin; HHb, deoxygenated haemoglobin and myoglobin; tHb, total haemoglobin and myoglobin [4]. Copyright © Reproduced with permission of the publisher. (Online version in colour.)

made it possible to image regional differences in skeletal muscle oxygenation and metabolism in different locations within a muscle. Some NIRS research groups have developed multi-channel NIRS devices and demonstrated spatial differences in oxygenation in exercising muscles [16,38,39]. Of particular interest are the recent findings that muscle contractions and even passive muscle stretch can result in a gradient of oxygen desaturation along the proximal–distal axis of a muscle [40,41]. Multi-channel NIR images from the quadriceps muscles during and after intermittent isometric knee-extension exercise are presented in figure 2 [4].

(*c*) *Portability*

Portable NIRS devices have been built to monitor human locomotion continuously and freely in the field [22]. Early models of portable NIRS devices were small, but still required external power supplies and a probe connected by wires to a control box attached to the person. New developments have led many manufacturers to produce wireless probes that can be attached to the muscles of interest with adhesive tape, with control modules that can either store various amounts of data or send signals to a nearby computer. For example, a wireless continuous-wave NIRS system, which is about the size of a cell phone, was developed by Artinis Medical Systems (the Netherlands). The device is used for measuring muscle and cerebral oxygenation during exercise [42]. A portable spatially resolved NIRS device has been developed by Astem Inc. (Japan). The device consists of a light-emitting diode (LED) light source, two photodiodes, microprocessors and a Bluetooth networking module. The total weight is 95 g and the size is $55 \times 77 \times 17$ mm. The feature of the system is correction of the influence of the subcutaneous fat layer on spatially resolved NIRS based on the theoretical analysis [43]. These devices allow unrestricted movements to be studied in a field or a clinical set-up.

(*d*) *Examples of recent advanced near-infrared spectroscopy application to medical sciences* (*post-2007*)

Since the review of NIRS application to medical sciences before 2007 can be found elsewhere [4], this section primarily deals with examples of advanced NIRS application later than 2007.

NIRS devices have been used to identify peripheral vascular disease (PVD). The time to half recovery of oxygen saturation showed reasonable, although not strong, agreement with the clinical assessment of PVD, and with some risk factors for cardiovascular disease [35]. The determination of resting muscle oxygen consumption using the venous occlusion method is suitable for clinical settings and allows non-invasive quantification of a compensatory peripheral adaptation in patients with PVD [44]. A review article evaluated the usefulness of ^{31}P-MRS and NIRS to determine mitochondrial function and the degree of ischaemia in type 2 diabetes mellitus and PVD, and found that the two measurements were well correlated for the assessment of mitochondrial function and the degree of ischaemia in these diseases (see [45] for review).

NIRS has been used to examine the effect of bronchodilators [46] and oxygen [47,48] administration on the improvement of attenuated oxygen delivery to the muscle in patients with chronic obstructive pulmonary disease (see [49] for review). NIRS has also been used to examine the effect of pharmacological treatment on blunted microvascular oxygen delivery to the muscle [50] and the effect of a rehabilitation programme on muscle oxygenation [51] in patients with chronic heart failure.

NIRS in combination with pulmonary oxygen uptake measurement was found to be useful for determining the status of peak capacity of oxygen extraction by skeletal muscles in patients with metabolic myopathies in an objective, quantitative and longitudinal manner [31,52]. Cardiorespiratory and muscle oxygenation responses to electrical stimulation-evoked leg cycling were measured using NIRS in patients with paraplegia [53]. The study observed that equilibrium

between oxygen demand and delivery was reached during prolonged electrical cycling, despite the lack of neural adjustment of leg vasculature in the paralysed lower limbs.

Deoxy-Hb/Mb kinetics was measured in patients with type 2 diabetes mellitus at the onset of moderate cycling exercise (see [45,54] for review). The study found that type 2 diabetic skeletal muscle demonstrated a transient imbalance of muscle oxygen delivery relative to oxygen uptake, suggesting a slower muscle oxygen blood flow increase.

NIRS was able to measure the increase in oxygenation of the resting neck/shoulder muscle in women with trapezius myalgia during cycling exercise [55] and to detect faster recovery of trapezius muscle reoxygenation 1 day after dry needling treatment in patients with chronic neck/shoulder pain [56]. NIRS in combination with microdialysis was applied to examine muscle oxygenation and intramuscular metabolites, respectively, in response to simulated repetitive work using a hand-held manipulandum in women with trapezius myalgia [57]. NIRS was also used to examine whether paraspinal muscle oxygenation during lumbar extension and flexion was improved after muscle relaxant administration in women with chronic low back pain [58].

4. What is the most exciting prospect for the next 20 years?

It is thought that embedding an NIRS device in exercise clothing, similar to those worn by speed skaters, can be used outside the laboratory with the help of energy-harvesting technologies (solar batteries, a light source with sunlight and band-pass filters) included in the suit, and could be used in a sporting event such as the Tour de France. We thought of a suit that gives the output of muscle oxygenation to a liquid-crystal panel such as a computer screen on the surface of the suit (figure 3). Each optical probe includes photo-detectors, a light source, signal-processing circuits, a microcontroller, power supply modules and wireless or storage units. Although the technology for data storage and small wireless probe sizes exists, the optical probe should have very low power consumption and be lightweight in order to measure data continuously during various exercises. A low-power system would be realized if ambient light through a band-pass filter can be used as a near-infrared light source. Energy-harvesting modules will become key devices for continual monitoring with this future mobile NIRS system. The issues related to power supplies and coordination among many source–detector pairs need to be resolved.

5. What is the major challenge to achieving this?

To build a running NIRS suit, the following technological and software inventions are needed.

(*a*) *Power supply with energy harvesting*

Recently, energy-harvesting power sources have been developed as a technology for ecosystem sensing [59]. The values of electrical power obtained from solar energy and vibration caused by motion are 10^{-4} and 10^{-3}–$10^{-4}\,\mathrm{W\,cm^{-2}}$,

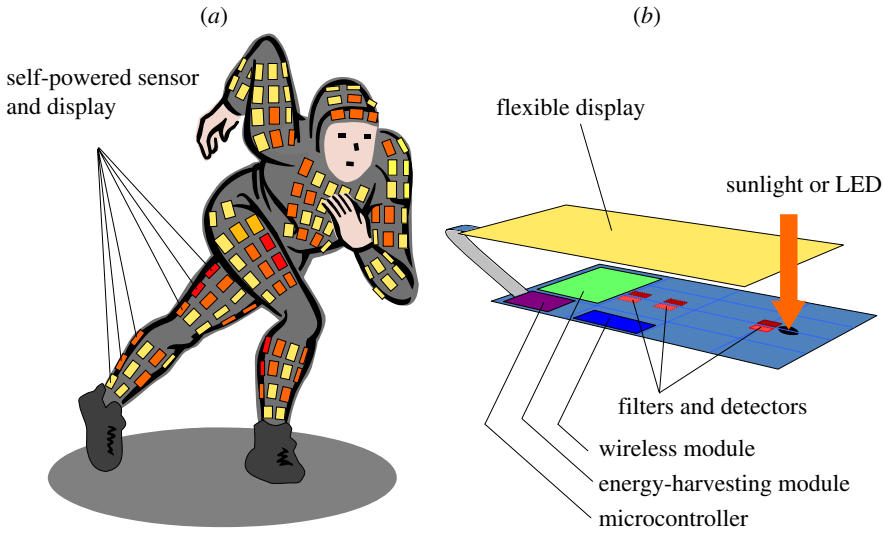

Figure 3. (*a*) A schematic of the NIRS imaging system. (*b*) Configuration of the measurement device, which consists of an energy-harvesting module, a microcontroller unit, a wireless module for real-time monitoring, detectors, band-pass filters for spectroscopy using sunlight and a flexible display for intelligible visualization. (Online version in colour.)

respectively. Therefore, the development of a high-efficiency energy-harvesting device is absolutely essential. A system for selecting the appropriate energy corresponding to the type of exercise and the ambient light intensity will be key.

(*b*) *Light source using sunlight and band-pass filters*

Light sources consume a substantial amount of electrical power of the NIRS system. The power required for generating an intermittent pulse by a 1 mW light source is 0.01–0.1 mW. If the sunlight is radiated through the 1×10 mm slit to the body surface, the 10^{-5} W light propagates to the tissue and the backscattered photon of 10^{-9} W is obtained at 10 mm from the light source. The intensity of the backscattered light would be converted to a voltage of 0.04 mV when the ratio of generated photocurrent to incident power is $0.4\ \mathrm{A\,W^{-1}}$, the feedback resistance of a current–voltage converter is 10 MΩ and the loss of NIR band-pass filters is 20 dB. The output voltage could then be used for measuring tissue oxygenation by designing a low-noise amplifier, signal-processing circuits and a high-resolution analogue-to-digital converter. However, interference by multiple light sources would occur if many optical probes are placed densely, because the sunlight to each probe cannot be controlled separately. Thus, methods of interference-free probe arrangement and a permeable optical shield are required. Even if natural power sources prove viable, it will still be necessary to ensure that changes in the ambient light intensity do not cause inaccurate measurements due to resultant instabilities in the power output.

(c) *Elimination of 'motion artefacts'*

Unwanted large spikes in the oxygenation chart sometimes occur when muscles are contracted strongly. The main factors contributing to the motion artefact are (i) a sub-millimetre gap between the sensor and the body surface and (ii) changes in pathlength for the muscle tissue due to changes in the shape of the muscle and in the thickness of a fat layer. The contact pressure between the optical probe and the skin should be monitored in order to reduce the influence of a small gap. To correct for these changes in pathlength, real-time measurements of the layered tissue structure are likely to be required using ultrasound or magnetic resonance imaging.

(d) *Measurement for deeper tissue*

The other big challenge is the extraction of signals propagated through the deep parts of the muscle tissue. There are many requirements for measuring signals from the deep parts and the surface of the muscle separately. There is no current robust and reliable way to achieve this, although methods using photoacoustic imaging [60] and optical computed tomography [61] are making progress. Real-time measurement of the tissue structure, as mentioned above, will be essential for the measurement of deeper tissues. The determination algorithm corresponding to each tissue structure based on large amounts of data from both experimental and theoretical studies will be needed. Three-dimensional oxygenation imaging is therefore likely to require a major breakthrough in methodology.

6. Conclusion

NIRS has been shown to be useful for the detection of changes in muscle metabolism and oxygen delivery in healthy subjects as well as in patients with various organ diseases and muscle-specific disorders. The major advances of the last 20 years have been: widespread availability of devices through the efforts of several commercial companies, the development of calibration approaches, the most robust being the physiological calibration using ischaemia and reperfusion, and the development of NIRS imaging and the start of truly portable devices. The use of NIRS devices has become practicable for both exercise and clinical use. However, along with applied clinical studies, basic research is still needed, such as the origin of the NIR signal (which fractions from arterioles, capillaries and venules, as well as from Hb and Mb), the NIR penetration depth or measurement area in tissue with varying source–detector arrangement (orientation) in the multi-layer model including the effect of non-muscular tissue, changes in optical properties during a wide range of tissue oxygenation status, varying subjects and exercise modality. Developing comfortable 'exercise' NIRS suits would be the ultimate goal for measuring oxidative metabolism in sport events and clinical sciences.

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