Oxidative metabolism in muscle

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SUMMARY

Oxidative metabolism is the dominant source of energy for skeletal muscle. Near-infrared spectroscopy allows the non-invasive measurement of local oxygenation, blood flow and oxygen consumption. Although several muscle studies have been made using various near-infrared optical techniques, it is still difficult to interpret the local muscle metabolism properly. The main findings of near-infrared spectroscopy muscle studies in human physiology and clinical medicine are summarized. The advantages and problems of near-infrared spectroscopy measurements, in resting and exercising skeletal muscles studies, are discussed through some representative examples.

1. INTRODUCTION

Oxidative metabolism is the dominant source of energy for skeletal muscle. The major physiological variables in O_2 utilization are expressed in the Fick equation: oxygen utilization ($\dot{V}O_2$) = (arteriovenous (a−v) O_2 difference) × (blood flow). The demand for O_2 is met by an increase in the O_2 delivery ($\dot{D}O_2$) and by an increase of O_2 extraction from oxyhaemoglobin (HbO_2). From rest to intense exercise, systemic a−v O_2 difference increases from about 5 to about 15 ml dl^{-1} and O_2 saturation of venous blood falls from 75 to 25 %. O_2 delivery by blood circulation is closely linked to the cardiac output and to the rate of muscle O_2 utilization during exercise (Wittenberg & Wittenberg 1989).

Although the use of optical methods, exploiting visible light (400–650 nm) to investigate muscle oxidative metabolism, dates back to 1937, when Millikan (Millikan 1937) demonstrated muscle deoxygenation on stimulation, its application is limited by the poor penetration depth. Conversely, near-infrared (650–1100 nm; NIR) spectroscopy (NIRS), a relatively new non-invasive technique, allows the simultaneous measurement of changes in intravascular (haemoglobin) and mitochondrial (cytochrome aa_3) oxygenation in 2–6 cm^3 of the limb muscles. Recent findings raise new arguments about the possibility of measuring muscle cytochrome aa_3 (Bradley 1996), while changes in the concentration of oxyhaemoglobin [HbO_2], deoxyhaemoglobin [Hb] and total haemoglobin ([Hbtot] = [HbO_2] + [Hb]) can be easily calculated by a modification of the Beer–Lambert law (Cope & Delpy 1988). In addition, with some simple physiological manoeuvres, it is possible to quantify muscle $\dot{V}O_2$ (Cheatle *et al.* 1991; De Blasi *et al.* 1993), flow (Edwards *et al.* 1993; De Blasi *et al.* 1994; Homma *et al.* 1996*a*) and venous saturation (Yoxall & Weindling 1996, 1997).

Various NIR optical techniques, differing in the type of light source (lamps, lasers, light-emitting diodes) and in the modality of the light sources (continuous wave, NIR_{CWS}; continuous wave spatially resolved, $NIRS_{SRS}$; pulsed, NIR_{TRS}; phase modulation, NIR_{PMS}) have been used for muscle studies. Although these studies reported interesting pathophysiological findings, it is still difficult to interpret the local metabolism properly due to the impossibility of distinguishing the origin of the signal. It is expected that the main contributor to the NIR signal is the venous haemoglobin. However, a recent study reported that, in cycling exercise, muscle oxygenation measurements by NIRS do not reflect the venous saturation (Costes *et al.* 1996).

The aim of this paper is to review the main findings of NIR muscle studies and to discuss, through some representative examples, the advantages and problems of NIRS measurement of resting and exercising human skeletal muscle.

2. NIRS STUDIES OF HEALTHY MUSCLES

The first NIR absorption spectrum of the human muscle was produced by Norris at the USA Department of Agriculture Research Service (Smith 1977). The spectral features of the haemoglobin, water and fat were identified and a chemometric algorithm was developed to quantify body fat (Conway *et al.* 1984). Myoglobin has similar absorption spectra to haemoglobin. However, the ratio of haemoglobin to myoglobin in human skeletal muscle is approximately ten (Seiyama *et al.* 1988).

In the Eighties several NIR_{CWS} prototypes were built (Jöbsis 1977; Giannini *et al.* 1982; Takada *et al.* 1987; Cope & Delpy 1988; Chance *et al.* 1988*a*, *b*; Hampson & Piantadosi 1988); forearm occlusion and exercise was used to test their instrumental capabilities. Most of the NIR muscle studies have been made using a low-cost continuous dual wavelength system (RUNMAN, NIM Inc., Pennsylvania, USA), which has recently been improved (Shiga *et al.* 1995). Although RUNMAN gives only relative values, a strong linear relationship was found between its values and forearm deep vein O_2 saturation during exercise (Mancini *et al.*

1994*a*). The RUNMAN has been used during various types of whole body upright exercise (e.g. treadmill, rowing and bicycling). Skeletal quadriceps oxygenation was investigated during constant and incremental work rate bicycle exercise (Wilson *et al*. 1989; Chance *et al*. 1992; Belardinelli *et al*. 1995*a*,*b*; Costes *et al*. 1996). It has been demonstrated that the rate of O_2 resaturation after exercise is faster in the endurance-trained athlete (rowers) than in sedentary controls (Chance *et al*. 1992). The effect of hypoxia on muscle oxygenation during arm exercise has been investigated (Jensen-Urstad *et al*. 1995). An interesting study on the effects of the increased sympathetic vasoconstrictor drive on muscle oxygenation in rhythmically contracting human forearm muscles was recently reported by Hansen *et al*. (1996).

Simultaneous application of ^{31}P magnetic resonance spectroscopy (MRS) with the RUNMAN provided a unique opportunity to investigate muscle metabolism and oxygenation (Kemp *et al*. 1994; Mancini *et al*. 1994*a*,*c*). The kinetics of reoxygenation paralleled those for regeneration of the high-energy compound phosphocreatine after submaximal exercise, and rapidly surpassed the metabolic processes after maximal effort (McCully *et al*. 1994*b*). Proton MRS and NIRS experiments also demonstrated that haemoglobin deoxygenation precedes myoglobin deoxygenation (Wang *et al*. 1990; Mancini *et al*. 1994*c*).

More quantitative muscle studies were performed using a three-wavelength NIR_{CWS} instrument (OM-100A, Shimadzu Co., Japan). This instrument was used in several studies, i.e. to develop a forearm $\dot{V}O_2$ method (Homma *et al*. 1996*a*), to investigate the influence of adipose tissue thickness on the NIR measurements (Homma *et al*. 1996*c*), to investigate skeletal quadriceps oxygenation during constant and incremental work rate bicycle exercise (Homma *et al*. 1993) and to study changes in muscle oxygenation during weight-lifting exercise (Tamaki *et al*. 1994). Quantitation was further improved by combining four-wavelength attenuation data, measured by the NIRO500 (Hamamatsu Photonics, Japan), with the optical path length that can be measured from separate NIR_{TRS} and NIR_{PMS} measurements during different conditions (Delpy *et al*. 1988; Chance *et al*. 1988*b*; Duncan *et al*. 1995*a*). Path length changes were less than 10% during arterial occlusion with maximal voluntary contraction (Ferrari *et al*. 1992, 1993; Duncan *et al*. 1995*b*). The NIRO500 was used in several studies, i.e. to develop forearm $\dot{V}O_2$ and flow methods (De Blasi *et al*. 1994), to investigate the effect of the treadmill speed and slope on the quadriceps oxygenation (Quaresima *et al*. 1995*b*, 1996*a*), and to study forearm muscle exercising simultaneously with various leg-cycling intensities (Bradley 1996).

3. NIRS MUSCLE STUDIES ON PATIENTS

The clinical significance of NIRS muscle findings in different diseases has been explored by several groups using different instrumentation. NIRS has been used to investigate the diseases associated with impaired tissue oxygenation, like heart failure and peripheral vascular disease (PVD). It has been shown that patients with congestive heart failure desaturate their muscle at lower work levels than normal subjects indicating an insufficient blood flow to the exercising muscles of these patients (Wilson *et al*. 1989; Mancini *et al*. 1994*c*; Belardinelli *et al*. 1995*c*; Matsui *et al*. 1995). The results indicate that NIRS can detect different muscle oxygenation profiles in patients with different levels of exercise intolerance.

Accessory respiratory muscle oxygenation during exercise was assessed on patients with heart failure and on heart transplant recipients (Mancini *et al*. 1991, 1994*b*, 1995, 1996).

Many studies were made to investigate PVD. Cheatle *et al*. (1991) found that at rest the $\dot{V}O_2$ of PVD patients was half of that found in healthy controls. A standardized treadmill exercise was used to investigate the calf oxygenation of patients with claudicatio intermittent (Komiyama *et al*. 1994; McCully *et al*. 1994*a*; Colier *et al*. 1995*a*). The patients with more severe impairment (i.e. insufficient O_2 delivery) presented an earlier decrease of muscle oxygenation. A delayed oxygenation recovery time after forearm occlusion was found in patients with chronic subclavian artery occlusion (Kurosawa *et al*. 1996). Tissue oxygenation was also investigated during electrically stimulated muscle contraction on patients with spinal cord injury (Monroe *et al*. 1995).

Muscle NIRS might also have a role in investigating the therapeutic efficacy of vasoactive substances. Recently, the effect of nifedipine (vasodilator) on the established effects of bryostatin (antineoplastic agent) was studied on the calf of patients with disseminated melanoma by combining ^{31}P MRS and NIR_{CWS} (Thompson *et al*. 1996). The assessment of the acute therapeutic effect of dobutamine on skeletal muscle oxygenation has been reported (Mancini *et al*. 1990).

Oxidative defects have been found in metabolic myopathies by NIR_{CWS} (Bank & Chance 1994; Chance & Bank 1995). The cytochrome *c* oxidase deficiency did not provoke any deoxygenation during calf exercise. This indicates an under-utilization of delivered O_2.

Recently, the muscle absorption and the reduced scattering coefficients were mapped on muscular dystrophy patients (Quaresima *et al*. 1996*b*). Using the same patients, the effect of the treadmill speed and slope on the quadriceps oxygenation was investigated (Quaresima *et al*. 1995*b*, 1996*a*,*c*). The Duchenne muscular dystrophy patients' performances were not O_2 limited.

Ageing might be associated with muscle blood flow reduction. It was found that elderly subjects had a longer rate of calf O_2 resaturation (McCully & Posner 1995).

4. ADVANTAGES AND LIMITATIONS IN THE USE OF MUSCLE NIRS

Figure 1 reports a typical example of the oxygenation changes in the resting muscle during ischaemia. Measurements were performed on the forearm by a four-continuous-wavelength instrument (NIRO500,

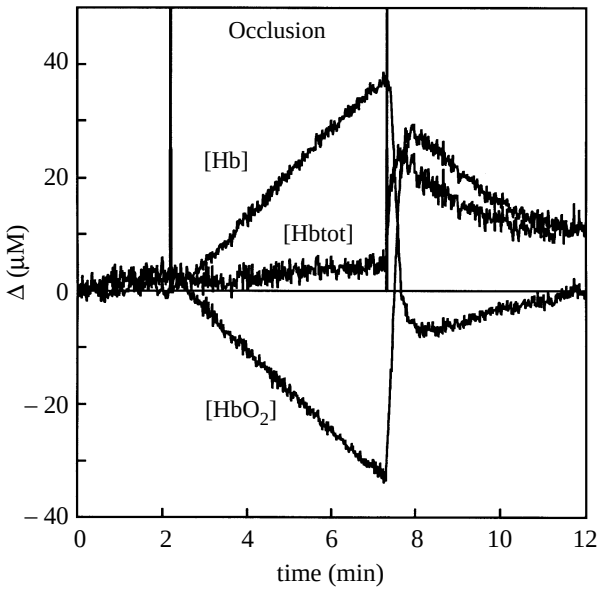

Figure 1. Effects of the vascular occlusion and release measured on the forearm by a NIR_{cws} instrument (NIRO500). Sampling time: 1 s.

Hamamatsu, Japan). Optodes were firmly positioned on muscle by a special support (Quaresima *et al.* 1995*a*). [Hb] and [HbO_2] changes are expressed as moles per litre of tissue. After 2 minutes of baseline an abrupt vascular occlusion was achieved by inflating a pneumatic cuff (240–260 mmHg). The occlusion was maintained for about five minutes. No consistent variations of [Hb] and [HbO_2] occurred during baseline, instead [HbO_2] decreased from the beginning of the occlusion. The [HbO_2] decrease was mirrored by [Hb] increase. No [Hbtot] variations occurred during the first two minutes of ischaemia. Instead [Hbtot] rose after cuff release. Hbdiff ([HbO_2]$^-$ [Hb]) is a good index of tissue oxygenation when no [Hbtot] changes occur. VO_2 can be calculated by measuring the rate of change of [HbO_2] to [Hb] (Cheatle *et al.* 1991). VO_2 can be calculated more precisely by the linear regression of the desaturation rate occurring during the first 60 s of ischaemia. The same method was applied by De Blasi *et al.* (1993, 1996*a*), Homma and Kagaya (1994) and Colier *et al.* (1995*b*) to measure VO_2 at rest and during isometric exercise. The recovery time, required from the cessation of vascular occlusion to resaturate the haemoglobin to 50%, can be calculated as described by Chance *et al.* (1992). This VO_2 method cannot be validated; however, another method using venous occlusion (De Blasi *et al.* 1994) has recently been validated by an invasive technique (Homma *et al.* 1996*a*). Both VO_2 optical methods provide comparable results. However, the venous occlusion method can be more easily repeated and is clinically more acceptable. This method also offers the advantage of measuring the blood flow (De Blasi *et al.* 1994) and mean tissue saturation (Sat) simultaneously (Yoxall & Weindling 1996, 1997). The blood flow method has been validated by plethysmography. Blood flow can be also estimated from changes in NIR signals from forearm muscle in response to the fractional inspired O_2 concentration (Edwards *et al.* 1993). All the methods using venous occlusion can be applied only at rest. These methods have been used to investigate forearm flow/VO_2 relationship on shock patients (De Blasi *et al.* 1996*b*).

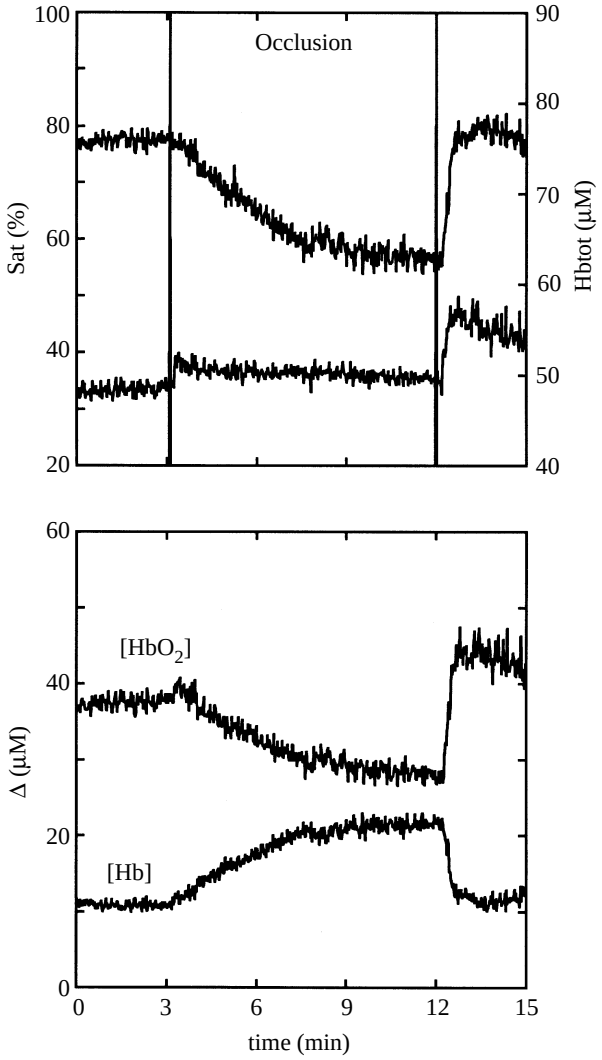

Figure 2. Effects of the vascular occlusion and release measured on the quadriceps by a NIR_{PMS} instrument (OMNIA). Sampling time: 4.8 s.

Figure 2 reports a typical example of a quadriceps oxygenation measurement performed on the resting muscle during thigh occlusion (450 mmHg) by a NIR_{PMS} instrument (OMNIA, ISS Inc. Urbana, Illinois, USA). This instrument has the advantage of measuring the absorption and scattering coefficients at two wavelengths and calculating the absolute values of [Hb] and [HbO_2] (De Blasi *et al.* 1995). From these it is possible to calculate Sat and [Hbtot]. A plateau was reached when Sat decreased by about 20%. This indicates that O_2 stores are not depleted. A similar finding was recently obtained using a portable NIR_{TRS} instrument (Liu *et al.* 1995; Hamaoka *et al.* 1996). Sat can also be measured by NIR_{SRS} instruments (Matcher *et al.* 1995*b*; Homma & Kagaya 1996*b*). Although Matcher found unexpected low Sat values at rest, Sat decreased by the same order of magnitude (about 30%) in ischaemia. The unexpected high saturation values found during prolonged ischaemia might be explained by several factors, such as (i) incomplete occlusion; (ii) absence of water and lipids contribution

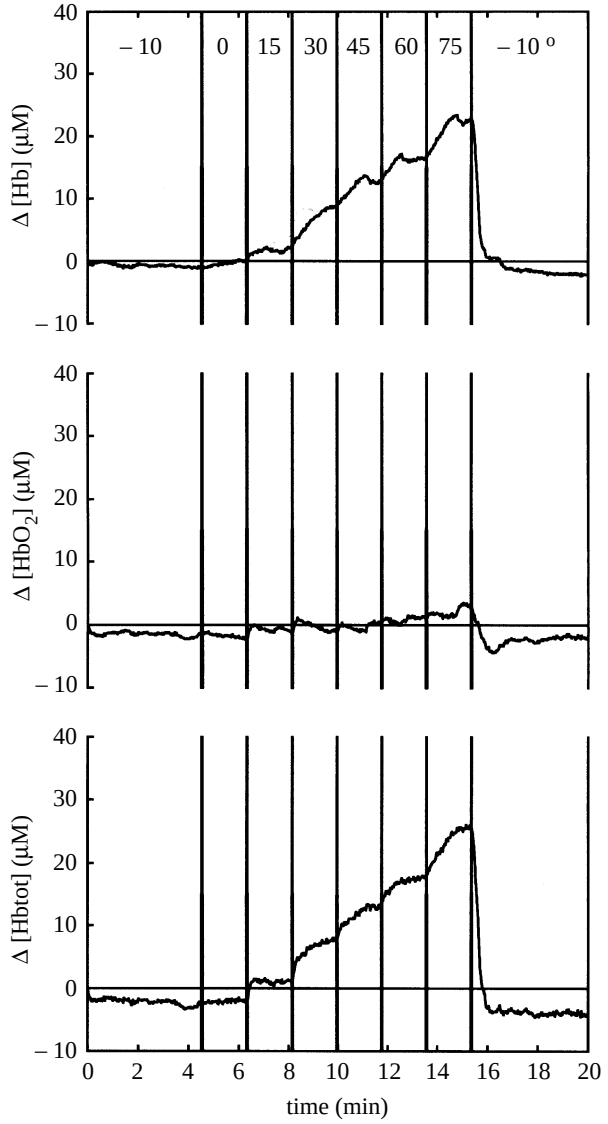

Figure 3. Effects of gravitational changes, at different angles of the tilting bed, measured on the calf by a NIR_{cws} instrument (NIRO500). Sampling time: 2 s.

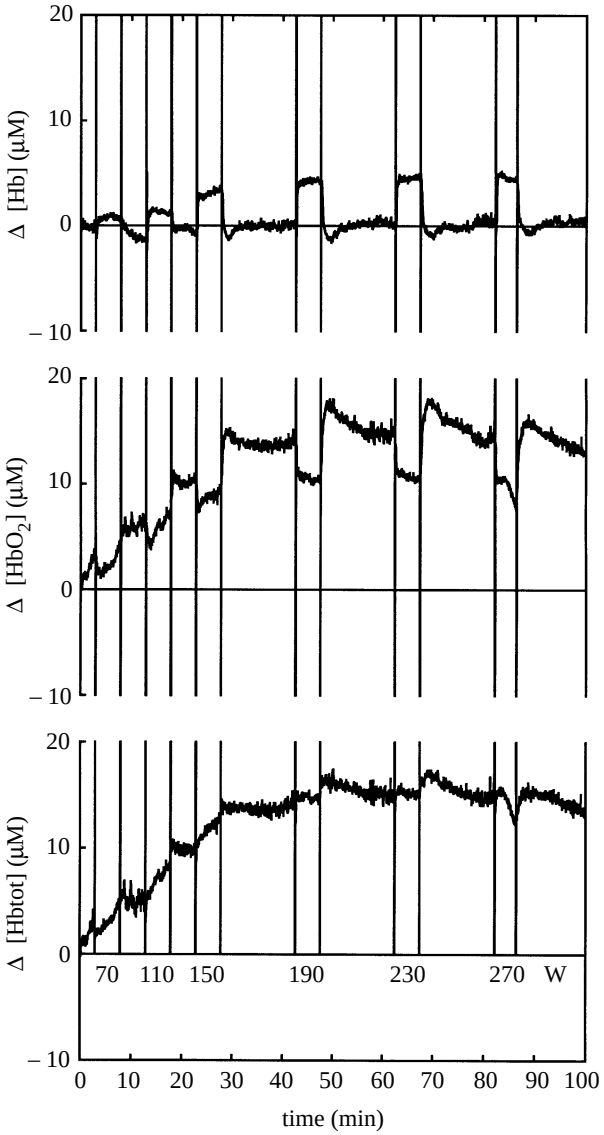

Figure 4. Effects of a series of square wave exercises on the quadriceps of an untrained cycling subject. Measurements performed with a NIR_{cws} (NIRO500). Sampling time: 2 s.

in the algorithm (Matcher *et al.* 1995*b*); (iii) wrong assumptions (homogeneous medium); and (iv) different diffusion of O_2 in the capillaries and veins. Although the reproducibility of this NIR_{PMS} instrument is satisfactory, the reliability of the data obtained from these instruments is still controversial. The NIR_{PMS}, NIR_{TRS} and NIR_{SRS} instruments offer such undoubted potential advantages that they should replace NIR_{CWS} instrumentation in the near future.

Figure 3 reports an example of the calf [Hb] and [HbO_2] changes during whole body passive postural variations (10° up to 75°, 15° increments). The subject was fixed on the bed to avoid any supplementary effort when the position was changed. Measurements were performed by a NIRO500. Negligible [HbO_2] changes occurred during the protocol; instead [Hb] consistently and gradually increased with the bed angle increments. [Hb] rapidly recovered to baseline values when the bed was repositioned at −10°. [Hbtot] gradually increased as well, and it reflected only the [Hb] changes. Considering that no $\dot{V}O_2$ changes are expected at rest, [Hbtot] rise (about 20 μM) is attributable to blood volume increases in the capacity of the venous vessels. This result suggests that postural changes should be taken into account in the interpretation of NIR findings and could be used to investigate the vascular compliance in healthy subjects and patients.

Figure 4 reports an example of increasing workloads. Measurements were performed on the quadriceps by a NIRO500. A series of square wave exercises performed on a cycloergometer were alternated with freewheel. The first exercise (70 W) provoked a rapid and gradual increase of [Hbtot]. The first four minutes were due to [Hb] rise, while the last minute was due to [HbO_2] rise. During the second exercise (110 W), [HbO_2] rise contributed more than [Hb] to the [Hbtot] increase. During the third exercise, the contribution to [Hbtot] rise was mainly due to [Hb] rise. In the following exercise steps [Hbtot] remained constant. During each freewheel recovery phase, [Hb] promptly returned to baseline values, while [HbO_2] reached different levels (each one higher than the previous one) up to 150 W.

Then [HbO_2] reached almost constant values. Therefore, the [Hbtot] tracing reflects mainly [HbO_2] variations, in particular after 190 W exercise when the muscle reached the maximal O_2 delivery. The 15 minute recovery time was not sufficient to recover [HbO_2] and [Hbtot] to baseline values. Assessment of muscle metabolism in these experimental conditions would require the calculation of blood flow and $\dot{V}O_2$. Unfortunately, non-invasive NIR optical methods are not applicable and invasive methods are expensive and uncomfortable. This represents a serious limit to the application of NIR in dynamic exercise.

A recent study (Costes *et al*. 1996) compared quadriceps oxygenation with the femoral venous O_2 saturation during steady-state cycling exercise at 80% of maximal O_2 uptake in normoxia and hypoxia (FiO_2 0.10). It was found that muscle oxygenation paralleled venous saturation in hypoxia, while it did not follow venous saturation in normoxia. Blood volume was not measured in this study. However, these data raise new questions about muscle oxygenation during steady-state exercise.

5. CONCLUSIONS

It has been shown that muscle NIRS can play a significant role in: (1) understanding healthy muscle oxidative metabolism; (2) understanding the effects of disease on muscle metabolism and function; (3) evaluating the efficacy of therapeutic intervention; and (4) confirmation of disease diagnoses.

Future muscle NIR studies should be combined with MRS techniques to probe the mechanisms further under various conditions. Unfortunately, this comparison is limited by the fact that MRS techniques are more restrictive with regard to the performance of repetitive motions within the bore of the magnet and currently require a confined horizontal position. The use of NIRS has become more widespread as cost and availability have improved.

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