Introduction

The purpose of this article is to highlight in vivo near-infrared spectroscopy (NIRS) studies for evaluating skeletal muscle oxygenation and oxidative metabolism, specifically in sport, health, and medical sciences. We present a brief background on the methodology, NIRS indicators for evaluating muscle energetic and physiology, and physiological and medical application. Detailed review articles recently published can be found elsewhere regarding the principles, limitations, and applications of NIRS in muscle exercise physiology and pathology1-3).

Since the early 1930’s the light in the visible region has been used for monitoring of changes in tissue oxygenation4). Thereafter, it was discovered that the mitochondrial NADH signal was detectable using the light during the electrical muscle stimulation, indicating the coupling of muscle contraction and mitochondrial activity5). Later, Jobsis (1977) discovered that the NIR light easily goes through the skull and set the stage for the recent application of NIRS to muscle physiology as well as brain oxygenation6). Chance et al. (1992) developed NIRS system that served as one of the first models to provide us with opportunities for further clinical muscle research in a noninvasive and portable way7).

Principles of Muscle NIRS Operation

Wavelengths of the NIR light ranges from 700 to 3000 nm, showing much less scattering and thus better penetration into biological tissue than visible light. However, light absorption by water limits the tissue penetration above 900 nm wavelength, leaving the 650 to 900 nm range. The major absorbing compounds of this wavelength region are intravascular hemoglobin (Hb), intramuscular myoglobin (Mb), skin melanin, and mitochondrial cytochrome c oxidase6). NIRS measurements rely on changes in O2 dependent absorption that occur in the heme, and copper containing compounds.

To calculate the changes in oxy-Hb/Mb, deoxy-Hb/Mb, or total-Hb/Mb, the equation of a two-, or multiple-wavelength method can be applied according to the following Beer-Lambert Law useing single-distance continuous wave light (NIRSDCWS).

\[
\Delta \text{OD} = -\log_e \left( \frac{I}{I_0} \right) = \varepsilon \text{PL} \Delta [C] \quad [1]
\]

\[
\Delta [C] = \frac{\Delta \text{OD}}{\varepsilon \text{PL}} \quad [2]
\]

where \(\varepsilon\) is the extinction coefficient (OD/cm/mM) (= constant), PL is the pathlength, \([C]\) is the concentration of absorber (mM), \(I\) is the detected light intensity, \(I_0\) is the incident light intensity, and OD is the optical density.

NIRSDCWS devices provide only the relative values of tissue oxygenation mainly due to the unknown path of NIR light through biological tissues. It is known that the penetration depth of the light into the tissue is approximately equal to half the distance between the light source and the detector9). Subcutaneous adipose tissue thickness greatly influences the light pathlength and makes it difficult to

Abstract

Near-infrared spectroscopy (NIRS) has been a useful method for the detection of changes in in vivo muscle oxygenation and oxidative metabolism in healthy subjects as well as in patients with various diseases. The advantage of using NIRS over other invasive techniques is that the device itself is more portable and the procedure can be performed more simply. So far, commercially available single-distance continuous wave NIRS (NIRS\text{SDCWS}) device provide only the relative values of tissue oxygenation mainly due to the unknown path of NIR light through biological tissues. In particular, subcutaneous adipose tissue thickness greatly influences the light pathlength and makes it difficult to quantify tissue oxygenation. Alternatively, the arterial occlusion and sensitivity correction approach using NIRS\text{SDCWS} and the pathlength determination using sophisticated time-resolved or phase-modulated spectroscopy could estimate the absolute value of tissue oxygenation. This paper aims at reviewing primarily NIRS\text{SDCWS} studies for evaluating skeletal muscle oxygenation and oxidative metabolism, specifically in physiological and medical research area.

Keywords: oxygen, noninvasive, exercise, muscle metabolism, clinical research, near-infrared
quantify tissue oxygenation, especially in the measurements of muscle oxygenation from the skin surface. A simple and common method for physiologically calibrating NIRSDCWS signals is to use the range of muscle oxygenation or deoxygenation caused by arterial occlusion followed by reactive hyperemia, which creates a 0-100% oxygenation or deoxygenation level. The arterial occlusion method is based on the assumptions that 5-6 minutes of ischemia will result in the complete disappearance of HbO₂ and that the reactive hyperemia after occlusion will almost completely eliminate deoxygenated Hb. The other correction method by NIRSDCWS is proposed such as the NIR signal sensitivity correction by measuring subcutaneous adipose tissue thickness. Using other optical approaches than NIRSDCWS, the pathlength of NIR light can be directly measured with time-resolved spectroscopy (NIRTRS) and phase modulation spectroscopy (NIRPMS). Therefore, the arterial occlusion and sensitivity correction approach using NIRSSDCW and the pathlength determination using sophisticated NIRTRS or NIRPMS could estimate the absolute value of tissue oxygenation.

**Application of Muscle NIRS to Physiological Science**

Several indicators such as muscle oxygenation level, reoxygenation recovery, and oxidative rate have been used for evaluating muscle function. Early studies used recovery kinetics of reoxygenation to evaluate oxygen consumption and delivery. Recovery time of muscle reoxygenation reflects a dynamic balance of oxygen delivery and oxygen demand in localized muscles. It is reported a prolonged recovery time, which suggested an increased energy deficit when rowing exercise intensity increased. They found a significant correlation between blood lactate and the recovery time of muscle reoxygenation after exercise. Recently, several studies reported that recovery time of muscle reoxygenation after submaximal to maximal exercise is one of the indicators for evaluating muscle oxidative capacity.

The effect of varying level of activity and/or inactivity was measured using NIRS. NIRS has also been used for evaluating training effects of exercise on muscle oxygenation and oxidative metabolism during endurance and sprint types of exercise. Six-week high-intensity interval training was evaluated during incremental exercise. The study reported that the rise in deoxygenation during incremental exercise was significantly higher after training, in comparison with before training, whereas the rise in total-Hb/Mb was not affected by training, suggesting an improvement in the O₂ extraction with training.

Muscle oxidative rate or oxygen consumption can be determined by monitoring the rate of muscle deoxygenation during a transient arterial occlusion. Changes in skeletal muscle oxidative function were measured by NIRS in disused forearm muscles evaluating the preventive effect of the endurance training protocol on deterioration of skeletal muscle. Muscle oxidative capacity was determined by the time constant for the recovery of mVO₂ applying repeated transient (5 to 10 sec) arterial occlusions after exercise. NIRS measurement detected the delayed mVO₂ recovery after exercise testing during immobilization. Therefore, NIRS provides useful information on non-invasive monitoring of deconditioning and reconditioning of skeletal muscle oxidative function.

**How We Estimate the Absolute Values of Muscle Oxygenation Using Near-Infrared Spectroscopy (NIRS)?**

- **Using Sophisticated Instruments**
  - Pathlength determination
    - NIR time-resolved spectroscopy (TRS) and phase-modulated spectroscopy (PMS)
  - NIRSDCWS + Magnetic resonance spectroscopy
  - O₂ store and resting metabolic rate quantification
  - NIRSDCWS only
    - Arterial occlusion method --- 0-100% level

- **NIR Sensitivity Adjustment**
  - Subcutaneous fat measurement

Fig. 1 The Approach for estimating the absolute value of muscle oxygenation using single-distance continuous wave (commercially available) near-infrared spectroscopy (NIRSDCWS).
Application of Muscle NIRS to Medical Science

Since the review of NIRS application to medical sciences before 2010 can be found in previous literature[1-3], this section primarily deals with examples of advanced muscle NIRS application later than 2010. Due to the strong dependence of skeletal muscle on oxidative metabolism during moderate intensity exercise, improvement of oxidative system of the body creates higher exercise performance. On the other hand, impairments of mVO₂ and/or oxygen delivery to working muscles will limit exercise performance leading to a functional deterioration. NIRS is suitable for measuring attenuation of mVO₂ and oxygen delivery in patients with various diseases and possesses greater advantages in portability and real-time monitoring over the conventional technologies.

NIRS has been used to evaluate skeletal muscle oxygenation in patients with heart disease. In particular, muscle oxygenation kinetics has been studied in patients with congestive heart failure (CHF)[4,20]. Recently, the effect of light-to-moderate-intensity aerobic exercise training was examined in patients with CHF[21]. The training-induced simultaneous improvement of oxygen uptake kinetics and vascular endothelial function, associated with an increase of peak peripheral oxygen extraction supports the concept of oxygen delivery impairment as a key factor determining exercise intolerance in CHF. The study was conducted aiming to determine whether prior exercise could increase muscle oxygenation and speed VO₂ kinetics during exercise in CHF[22]. The study found that prior moderate-intensity exercise improves muscle oxygenation and speeds VO₂ kinetics in mild CHF. However, the most severely limited patients appear to have an intramuscular pathology that limits VO₂ kinetics during moderate exercise.

Patients with chronic obstructive pulmonary disease (COPD) frequently develop skeletal muscle and vascular abnormalities as complications of their disease[23,24]. Recently, NIRS has been used to examine the effect of bronchodilators administration on the improvement of lowered oxygen delivery to the muscle in patients with COPD[25]. The study was conducted to investigate the influence of skeletal muscle oxygenation on VO₂ during exercise in patients with COPD[26]. VO₂ is highly influenced by oxygen utilization in exercising muscles, as well as by blood oxygenation levels and cardiac function.

NIRS measurements have been used to study patients with neuromuscular disorders. In an early study, an increase in muscle oxygenation (paradoxical oxygenation) at the onset of treadmill exercise has been primarily reported in patients with cytochrome c oxidase deficiency[27]. Recently, the study addressed whether O₂ delivery during recovery from high-intensity, supra-gas exchange threshold exercise would be matched to O₂ utilization at the microvascular level in patients with mitochondrial myopathy[28]. They found that the slower rate of recovery of muscle metabolism after high-intensity exercise is not related to impaired microvascular O₂ delivery in patients with mitochondrial myopathy, indicating the intra-myo-cyte or oxidative metabolism abnormalities.

Several studies have used the rate of recovery of re-oxygenation for evaluating DO₂ to the calf muscles in patients with peripheral arterial disease (PAD)[29,30]. The rationale for using NIRS is an extensive study, which reported a good agreement between faster PCr recovery kinetics and faster oxygenation kinetics measured with NIRS[29]. A consistent finding with NIRS measurements in PAD patients is slower rates of calf reoxygenation after exercise[29,30]. Recently, a randomized trial was performed to determine if there were differences in calf muscle StO₂ parameters in patients before and after 12 weeks of a traditional walking or walking-with-poles exercise program[31]. Tissue oxygenation decline during treadmill testing was less for patients assigned to a 12-week traditional walking program when compared to those assigned to the 12-week walking-with-poles program. The study examined a novel diffuse correlation NIRS for monitoring of blood flow and oxygenation changes in skeletal muscles during arterial revascularization (via bypass grafts or percutaneous transluminal angioplasty)[32]. The acute elevations/improvements in calf muscle blood flow after intervention were associated with significant improvements in symptoms and functions after arterial revascularization. In general, the investigation corroborates potential of the optical methods for objectively assessing the success of arterial revascularization.

Muscle oxygenation and metabolism were tested using NIRS in children with end-stage renal disease (ESRD) before and after renal transplantation during submaximal hand-grip exercise[33]. The mVO₂ and reoxygenation recovery time after exercise significantly improved after renal transplantation. The study examined the potential for vascular and metabolic dysfunction in patients with renal failure and the effect of handgrip exercise training on forearm vasodilator responses with NIRS in patients receiving hemodialysis[34]. Vasodilator response was significantly smaller in the renal failure patients compared with those in the controls. These studies show that NIRS is able to detect muscle hypoperfusion in patients with renal failure as well as the functional alterations of muscle oxidative metabolism that occur after renal transplantation.

The results of medical NIRS application suggest that NIRS is a promising tool for monitoring non-invasively the metabolic impairment in the settings of follow-up and in the assessment of therapies and interventions.

Conclusion

NIRSDCWS has been a useful commercially available tool for the detection of changes in muscle oxygenation and oxidative metabolism in healthy subjects as well as
in patients with various diseases. The advantage of using NIRSDCWS over other invasive techniques is that the device itself is more portable and the procedure can be performed more simply. So far, NIRSDCWS devices provide only the relative values of tissue oxygenation mainly due to the unknown path of NIR light through biological tissues. In particular, subcutaneous adipose tissue thickness greatly influences the light pathlength and makes it difficult to quantify tissue oxygenation. Alternatively, the arterial occlusion method, pathlength determination using TRS or PMS, and sensitivity correction approach could estimate the absolute value of tissue oxygenation.

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References


25) Berton DC, Barbosa PB, Takara LS, Chiappa GR, Siqueira AC, Bravo DM, Ferreira LF and Neder JA. 2010. Broncho-
dilators accelerate the dynamics of muscle $O_2$ delivery and utilisation during exercise in COPD. *Thorax* 65: 588-593.


