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Bruno Grassi
Valentina Quaresima

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REVIEW

Near-infrared spectroscopy and skeletal muscle oxidative function *in vivo* in health and disease: a review from an exercise physiology perspective

Bruno Grassi[a,b,*] **and Valentina Quaresima**[c]
[a]University of Udine, Department of Medical and Biological Sciences, Piazzale M. Kolbe 4, Udine, I–33100 Udine, Italy
[b]Institute of Bioimaging and Molecular Physiology, National Research Council, I-20030 Segrate, Milan, Italy
[c]University of L'Aquila; Department of Life, Health and Environmental Sciences, Via Vetoio, I-67100 L'Aquila, Italy

Abstract. In most daily activities related to work or leisure, the energy for muscle work substantially comes from oxidative metabolism. Functional limitations or impairments of this metabolism can significantly affect exercise tolerance and performance. As a method for the functional evaluation of skeletal muscle oxidative metabolism, near-infrared spectroscopy (NIRS) has important strengths but also several limitations, some of which have been overcome by recent technological developments. Skeletal muscle fractional O_2 extraction, the main variable which can be noninvasively evaluated by NIRS, is the result of the dynamic balance between O_2 utilization and O_2 delivery; it can yield relevant information on key physiological and pathophysiological mechanisms, relevant in the evaluation of exercise performance and exercise tolerance in healthy subjects (in normal and in altered environmental conditions) and in patients. In the right hands, NIRS can offer insights into the physiological and pathophysiological adaptations to conditions of increased O_2 needs that involve, in an integrated manner, different organs and systems of the body. In terms of patient evaluation, NIRS allows determination of the evolution of the functional impairments, to identify their correlations with clinical symptoms, to evaluate the effects of therapeutic or rehabilitative interventions, and to gain pathophysiological and diagnostic insights. [DOI: 10.1117/1.JBO.21.9.091313]

Keywords: near-infrared spectroscopy; skeletal muscle bioenergetics; skeletal muscle oxidative metabolism; exercise physiology; exercise tolerance; review.

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1 Introduction

Near-infrared (NIR) spectroscopy (NIRS) applied to skeletal muscle has often been seen with some skepticism by many exercise physiologists. Once at a scientific meeting, one of the authors of the present review was told by Dr. Bengt Saltin (one of the most influential exercise physiologists of the last decades): "Unless you manage to measure O_2 consumption, this technique has not much physiological interest." We respectfully disagree with Dr. Saltin's statement. Although we recognize that NIRS has several limitations, some of which have been overcome by recent technological developments, the method also has important strengths and can give valuable (and noninvasive) functional insights into skeletal muscle oxidative metabolism *in vivo* during exercise, in health and disease.

At this purpose, the present review has been devoted, from an "exercise physiology point of view," to discuss some of the main issues related with the role of NIRS in the functional assessment of oxidative metabolism in skeletal muscles during exercise, with specific attention to integrative aspects and to the factors limiting exercise tolerance. Attention will also be paid to studies carried out in diseased populations, as well as to some recent and exciting technical and methodological developments.

2 Oxidative Metabolism During Exercise and Tools of Investigation

During supramaximal exercise lasting up to a few seconds, the metabolic power output in humans can increase by about 150 to 200 times compared with the value observed at rest. Among the tissues of the body, only skeletal muscle can sustain such extraordinary increases in metabolic power output, which is made possible by the splitting of high energy phosphates present in muscle as adenosine triphosphate (ATP) and creatine phosphate (PCr). If the exercise is carried out for longer periods of time (up to several minutes, or even hours), the maximal metabolic power output, which can be sustained, decreases hyperbolically as a function of the duration of the exercise[1–4] and oxidative metabolism becomes the prevalent, or substantially the only, mechanism responsible for ATP resynthesis. In other words, in most of our daily activities, either related to work or leisure, the energy for muscle work substantially comes from the oxidation of glucose and lipids in muscle fibers, culminating in oxidative phosphorylation at the mitochondrial respiratory chain.

It is not surprising, then, that the maximal power by oxidative metabolism and the fraction of this power, which can be sustained for relatively long periods of time, are intrinsically related to exercise performance and tolerance. An impairment of

*Address correspondence to: Bruno Grassi, E-mail: bruno.grassi@uniud.it

oxidative metabolism, which occurs in several types of patients, inevitably leads to a reduced exercise tolerance, which may represent one of the main determinants of the clinical picture and quality of life, as well as an important predictor of mortality.[5]

The availability of reliable tools to investigate *in vivo*, with precision and reproducibility, muscle oxidative metabolism, possibly noninvasively and with a reasonably elevated temporal resolution, appears therefore of utmost importance. This applies to athletes (sports activities), healthy subjects (both in normal and in altered environmental conditions), and patients affected by many diseases. In the patients, these tools would allow the follow-up of the impairments with time, the identification of potential correlations with clinical symptoms, the evaluation of the effects of therapeutic or rehabilitative interventions, and could also yield pathophysiological and diagnostic insights.

Whole body oxygen consumption ($\dot{V}O_2$) can be measured with relatively good precision (variability of measurements of about 5% in the best laboratories) at the mouth of the subject or at the alveolar level[6] (pulmonary $\dot{V}O_2$, $\dot{V}O_{2p}$) by the classic open circuit method. These measurements have been carried out in innumerable studies and lead to the definition of concepts like the maximal aerobic power ($\dot{V}O_{2p,max}$), which evaluates the integrated performance of the respiratory, cardiovascular, and skeletal muscle components of the O_2 pathway from ambient air to the mitochondria of skeletal muscles. The availability of methods to determine $\dot{V}O_{2p}$ with an elevated temporal resolution ("breath-by-breath" measurements)[6–8] expanded the spectrum of the functional evaluation variables and allowed the introduction of concepts such as the "gas exchange threshold,"[9] the "critical power,"[1] or the various phases of the $\dot{V}O_2$ "kinetics."[3,4,6,10] The main limitation of the whole body $\dot{V}O_{2p}$ measurements resides in the impossibility to discriminate between the exercising muscles and the rest of the body, as well as between different muscles engaged in the exercise. Moreover, the presence of O_2 stores between the site of measurement (the mouth or the alveoli) and the sites of gas exchange at the skeletal muscle level complicates data interpretation during metabolic transitions.[6] A further limitation derives from the diffusion of automated and apparently easy-to-use "metabolimeters," which led to the proliferation of data and studies of dubious quality.

The relationship between $\dot{V}O_2$, blood flow and O_2 extraction can be expressed by the Fick equation, based on the principle of mass conservation. If applied "across" a single muscle or a single muscle group, the equation reads as follows:

$$\dot{V}O_{2m} = \dot{Q}_m * [C(a\text{-}v)O_{2m}], \quad (1)$$

in which $\dot{V}O_{2m}$ represents muscle $\dot{V}O_2$, $\dot{Q}_m$ muscle blood flow, and $C(a\text{-}v)O_{2m}$ arterial-venous O_2 concentration difference across the muscle.

By rearranging Eq. (1):

$$C(a\text{-}v)O_{2m} = \dot{V}O_{2m}/\dot{Q}_m. \quad (2)$$

$\dot{V}O_{2m}$ measurements have been carried out with a high temporal resolution also during metabolic transitions in humans[11,12] or in isolated *in situ* animal models.[13,14] The main limitations of this approach can be summarized as follows: invasiveness of the measurements; technical problems inherently associated with *in vivo* blood flow measurements; the substantial impossibility (in human studies) to sample venous blood directly effluent from the exercising muscle, with a resulting "contamination" of venous blood coming from other muscles.

Alternative methods allowing a functional evaluation *in vivo* of skeletal muscle oxidative metabolism derive from the utilizations of phosphorus (^{31}P) or proton (^{1}H) nuclear magnetic resonance spectroscopy (^{31}P-MRS, and ^{1}H-MRS, respectively). As far as ^{31}P-MRS, the method has been utilized to determine the kinetics of recovery of muscle [PCr] following exercise. After assuming the equilibrium of the creatine kinase (CK) reaction, the rate of recovery of [PCr] is a function of mitochondrial ATP production, and therefore it can be considered a tool for evaluating the function of skeletal muscle oxidative metabolism.[15] ^{1}H-MRS, on the other hand, detects myoglobin (Mb) desaturation, thereby allowing an estimation of intracellular PO_2.[16,17] These technologically formidable methods are intrinsically limited by the very high cost of the instrumentation and by the strict constraints on the exercise modality determined by the size of the bore of the magnet.

In short, the above mentioned methods, although widely utilized, have intrinsic limitations. Therefore, noninvasive, precise, reproducible, and relatively low-cost functional evaluation tools to be utilized *in vivo*, characterized by an elevated temporal resolution, allowing the discrimination between (and possibly within) skeletal muscle groups, would be needed. Does NIRS satisfy, at least in part, these needs? The following sections of the present review will deal with this issue.

3 Near-Infrared Spectroscopy from an Exercise Physiology Point of View

Several excellent review articles about the application of NIRS in skeletal muscle have been published.[18–25] These reviews carry the relevant information on principles, methods, instruments, and so on. Ferrari et al.[24] included a useful list of practical recommendations. The present review article has been specifically devoted to discuss, from an "exercise physiology point of view," some of the main issues related with the role of NIRS in the functional evaluation *in vivo* of oxidative metabolism in skeletal muscles, within an integrated perspective related to exercise tolerance. A specific attention will be paid to NIRS studies carried out in diseased populations, as well as to some recent and exciting technical and methodological developments. The latter testify how NIRS, as a tool of functional investigation of skeletal muscles, is still in a steep portion of a gain in knowledge versus time curve. The role of NIRS in evaluating oxidative skeletal muscle performance in sport activities and in athletes, which would deserve a dedicated review article, is not covered in the present article. Likewise, the effects of exercise on brain hemodynamics, as measured by NIRS,[26,27] as well as the role of NIRS in the evaluation of brain function[21,25] are not discussed.

3.1 *Tissue Interrogated by the Near-Infrared Spectroscopy Probe*

When the probe is applied on the skin overlying a muscle of interest, NIRS instruments can interrogate only a relatively small (2 to 6 cm^3) and superficial volume of skeletal muscle tissue.[28] It is generally accepted that the depth of penetration of the NIR light in tissues roughly corresponds to half of the distance between the light source and the detector (which is usually between 3 and 5 cm). This intrinsic technical limitation makes the measurement of the thickness of the skin and

subcutaneous adipose tissue layer, at the site of placement of the NIRS probe, mandatory. The measurement can be carried out by a caliper or, more precisely, by ultrasound.[29,30] The recent availability of low-cost hand-held ultrasound devices has significantly facilitated these measurements. Although no cutoff values are recognized as standard, a value greater than ~20 mm would presumably make NIRS measurements, as carried out by standard instruments, rather meaningless in terms of investigating skeletal muscle. This precludes the utilization of the method in subjects with a relatively thick layer of subcutaneous fat, such as obese patients or in patients with significant muscle atrophy.

Table 1 Main commercial muscle NIRS oximeters.

Device	Company, country	Technique	Type of sources	N. of wl	Time-resolution (Hz)	No. of channels	Measurable parameters	Approvals	Websites
Oxymon Mk III[a]	Artinis, The Netherlands	Multidistance CW	laser	2	250	2	SO_{2m}		www.artinis.com
Exercise Monitor Hb11[b]	Astem, Japan	Multidistance CW	LED	2	1	1	SO_{2m}		www.astem-jp.com
Fore-sight Elite®[a]	CAS, Inc., United States	Multidistance CW	LED	5	0.5	4	SO_{2m}	FDA, CE	www.casmed.com
NIRO-200 NX[a]	Hamamatsu, Japan	Multidistance CW	LED	3	20	2	SO_{2m}	CE	www.hamamatsu.com
tNIRS-1[a]	Hamamatsu, Japan	TD	laser	3	0.2	2	SO_{2m}, atHb	CE	www.hamamatsu.com
OxiplexTS™[a]	ISS, United States	Multidistance FD	laser	2	50	2	SO_{2m}, atHb		www.iss.com
O3™ Regional Oximetry[a]	Masimo, United States	Multidistance CW	LED	4	1	up to 6	SO_{2m}, SO_{2p}	CE	www.masimo.com
INVOS™ 5100C[a]	Medtronic, United States	Multidistance CW	LED	2	0.2	up to 4	SO_{2m}	FDA	www.medtronic.com
moorVMS-NIRS	Moor, United Kingdom	Multidistance CW	LED	2	5	2	SO_{2m}	CE	www.moor.co.uk
NIMO[ab]	Nirox, Italy	Multidistance CW	laser	3	40	up to 4	SO_{2m}	CE	www.nirox.it
SenSmart™ Model X-100[a]	Nonin, United States	Multidistance CW	LED	4	0.6	up to 5	SO_{2m}, SO_{2p}	FDA	www.nonin.com
BOM-L1 TR SF	Omega-wave, Japan	Single distance	laser	3	1	1	SO_{2m}		www.omegawave.co.jp
Cerox™ 3210F[a]	Ornim, Inc., United States	Multidistance CW	laser	3	0.33	up to 4	SO_{2m}, flow[c]	FDA	www.ornim.com
CareGuide™ 4100	Reflectance, Med., United States	Multidistance CW	LED	Multiple	0.03	1	SO_{2m}, pHm	FDA	www.reflectancemedical.com

Note: atHb, absolute total hemoglobin concentration; CE, European conformity marking as a medical device; CW, continuous-wave spectroscopy; FD, frequency-domain spectroscopy; FDA, Food and Drug Administration's approval; LED, light emitting diodes; N., number; pHm, muscle pH; SO_{2m}, muscle oxyhemoglobin saturation; SO_{2p}, peripheral arterial oxyhemoglobin saturation (measured by pulse oximetry); TD, time-domain spectroscopy; wl, wavelengths.
[a]Brain oximeter utilized also in muscle studies
[b]Oximeter with fat-layer compensation
[c]Cortical microcirculation blood flow measurement using Doppler shift in coherent light signals.

The fact that the NIR light has to cross the skin and subcutaneous fat in order to reach the underlying skeletal muscle tissue inevitably causes a sensitivity problem and represents a "contamination" of the signal of interest, which is the one coming from skeletal muscle. The problem can be particularly significant in conditions in which skin blood flow increases during exercise for thermoregulatory purposes (see also below). Several "correction algorithms" have been proposed (see the discussion in Ferrari et al.[24]), which, however, are not included in most of the commercial instruments (Tables 1 and 2). Ohmae et al.[31] have recently described a simple method, which does not require ultrasound, for the sensitivity correction for the influence of fat thickness on muscle oxygenation, based on the estimation of fat thickness by time-domain NIRS (TD-NIRS) (see below). van Beekvelt et al.[32] and Koga et al.[29] proposed other correction algorithms based on the assumption that resting $\dot{V}O_{2m}$ [estimated on the basis of the concentration increases in deoxy-hemoglobin (Hb) and deoxy-myoglobin (Mb), [deoxy(Hb+Mb)]) after applying a cuff occluding the arterial circulation, see below] would be inversely correlated with skin + subcutaneous tissue thickness. Another approach, based on [total(Hb+Mb)] values obtained at rest by quantitative multichannel TD-NIRS, has been proposed.[33]

In the quadriceps muscle (often investigated by NIRS studies) and in other muscles as well, deeper muscle regions are characterized by a greater proportion of oxidative fibers compared to superficial regions[34] and by a more oxidative energy metabolism. Accordingly, the deeper fibers have a greater sensitivity toward vasodilatory control mechanisms.[35] An anatomical "gradient" in fiber type is presumably associated with a gradient also in terms of metabolism and blood perfusion.[36] This has been substantially confirmed, in the quadriceps muscle of humans, in studies carried out by utilizing a "high-power" TD-NIRS instrument,[37,38] which allows interrogation of slightly deeper portions of muscle. This instrument, which delivers NIR light with a power about 30 times higher than that of traditional NIRS instruments, allows an effective mean penetration depth of about 3 cm and may represent a substantial technical improvement.

Another problem related to the small volume of tissue interrogated by conventional NIRS instruments derives from the fact that muscle activation and metabolism,[39] and even more markedly so blood perfusion[36,40,41] are heterogeneously distributed within and between exercising muscles. Excellent reviews on metabolic and blood flow heterogeneities in skeletal muscle, in health and disease, have been recently published.[42,43] Since skeletal muscle fractional O_2 extraction (see below) can increase during exercise only three to four times above the values at rest, whereas $\dot{V}O_{2m}$ can increase 100-fold, directing blood flow according to O_2 needs is crucial to allow sustained contractile performance.[43]

Most studies (but not all[44]) found good matching between muscle O_2 delivery ($\dot{Q}O_{2m} = \dot{Q}_m * CaO_2$, in which CaO_2 represents the arterial O_2 content), or substrates delivery, and $\dot{V}O_{2m}$ between macroscopic (volume of several cm^3) regions of a muscle, or between different muscles involved in the exercise.[30,45,46] However, when the resolution capacity of the measurements went down to about 1 cm^3, the spatial matching between $\dot{Q}O_{2m}$ and $\dot{V}O_{2m}$ (assessed via PCr depletion) was far from being perfect.[45] The presence (or absence) of a matching between the two variables could be more functionally relevant at a microscopic level. According to Segal,[47] the recruitment of contiguous "microvascular units," whose volume is orders of magnitude smaller than the volume investigated by NIRS, could be difficult to match with the recruitment of motor units, whose fibers are sparse within the muscle. In other words, blood flow cannot specifically increase to a specific muscle fiber or to the fibers of a motor unit,[47] but must rather

Table 2 Main commercial continuous wave portable/wearable NIRS systems for muscle studies with wireless data transmission.

Device	Company, country	Technique	N. of wl	Time-resolution (Hz)	No. of channels	Measurable parameters	Websites
OctaMon[a]	Artinis, The Netherlands	Single-distance	2	50	8	O_2Hb/HHb changes	www.artinis.com
PortaMon Mk II[b]	Artinis, The Netherlands	Multidistance	2	10	1	SO_{2m}	www.artinis.com
BSX Insight 2.0	BSX Athletics, LLC, United States	n.a.	n.a.	n.a.	1	SO_{2m}, LT	www.bsxinsight.com
PocketNIRS Duo®[c,d]	DynaSense Inc., Japan	Single-distance	3	60	2	O_2Hb/HHb changes	www.dynasense.co.jp
Moxy[e]	Moxy Monitor, United States	Monte Carlo modeling	4	2	1	SO_{2m}	www.moxymonitor.com
MyNimo	Nirox, Italy	Multidistance	3	10	1	SO_{2m}, atHb	www.nirox.it
OxiTor-M2	Pathonix Innovation Inc., Canada	Multidistance	2	30	1	SO_{2m}	www.pathonix.com

Note: atHb, absolute total hemoglobin concentration; HHb, deoxyhemoglobin; LT, lactate threshold evaluation by SO_{2m} tracing; n.a., not available; N., number; O_2Hb, oxyhemoglobin; SO_{2m}, muscle oxyhemoglobin saturation; wl, wavelengths.
[a]Muscle imager.
[b]Accelerometer is available on request.
[c]Commercially available only in Japan.
[d]Smartphone controllable system.
[e]Water resistant case.

increase over a relatively wide region of the muscle, leading to the possibility of "over-perfusing" inactive or relatively inactive fibers.

In any case, the apparently good matching between $\dot{Q}O_{2m}$ and $\dot{V}O_{2m}$ in different macroscopic areas of the same muscle, or of different muscles,[45,46,48] appears to be difficult to reconcile with the macroscopic heterogeneity of muscle deoxygenation observed by several studies.[29,37,49] The issue, in our opinion, is not settled, and further studies with higher spatial resolution devices are needed.

In summary, the area of muscle investigated by the NIRS probe may not represent, in terms of fiber types, fiber activation and the matching of $\dot{V}O_{2m}$ and $\dot{Q}O_{2m}$, a reliable picture of the situation in the whole muscle. In this respect, however, it should be considered that the same limitation, frequently overlooked, is intrinsically associated with other diffusely utilized methods, which investigate only a small and relatively superficial portion of a muscle, such as muscle biopsy.

3.2 *Where do the Near-Infrared Spectroscopy Signals Come From?*

The NIRS signals are the result of the weighted average of the O_2 saturations of the heme groups of Hb in the vascular bed (small arteries, arterioles, capillaries, venules, small veins) and of the Mb heme group in muscle fibers. In terms of Hb, most of the signal comes from small vessels, because larger arteries and veins (greater than ~1 mm in diameter) have very high heme concentrations, which absorb all the NIR light. Although it is often considered that venous–venular compartments represent the majority of blood present in skeletal muscle,[19,20] direct measurements suggest that this may not be the case. According to Poole and Mathieu-Costello,[50] capillaries would contribute to >90% of the total blood volume in muscle. According to these data, the storage of blood in veins would mainly occur externally to the muscle. Thus, the vascular component of the NIRS signals would predominantly come from the capillaries. Since in normal conditions, all regions of the muscle receive nearly-fully oxygenated arterial blood, oxygenation changes detected by NIRS would mainly reflect changes in capillary (Hb-related) and intracellular (Mb-related) O_2 levels.

A relative controversy exists about the role played in NIRS signals by the heme group in Mb. It has been traditionally assumed that Hb is responsible for most of the overall NIRS signal.[51,52] The concept is supported by the observation of strong correlations, observed in different experimental models, between muscle oxygenation values obtained by NIRS and O_2 saturation in venous blood (see below). Studies carried out by NIRS in combination with ^{1}H-MRS, aimed at distinguishing between Hb and Mb desaturation during ischemia and exercise, yielded conflicting results.[53,54] According to modeling papers,[55,56] Mb may contribute to ≥50% of the total [Hb+Mb] NIRS signal. This concept is supported by a study in which the contribution of Hb and Mb to *in vivo* optical spectra was determined by wavelength shift analysis.[57]

The relative role of Hb versus Mb is likely different at rest and during exercise. As discussed above, most of the intravascular NIRS signals likely come from the capillaries. In resting conditions, capillary hematocrit can be significantly lower than that in the systemic circulation.[58] During exercise, on the other hand, capillary hematocrit increases, reaching values that are not substantially different from those in larger vessels.[58] Thus, the role of Hb (versus that of Mb) in determining the NIRS signals would be higher during exercise compared to rest.

The possibility to separate Hb and Mb desaturation could give valuable insights into the mechanisms regulating peripheral O_2 diffusion and $\dot{V}O_{2m}$. For example, by applying NIRS on a rat model of isolated hindlimb, perfused with Hb-free well-oxygenated Krebs-Henseleit buffer, Takakura et al.[59] isolated the signal deriving from Mb desaturation and could calculate the intracellular PO_2, making inferences on the role of Mb in O_2 supply to mitochondria at exercise onset.

In any case, the whole diffusion pathway, including vascular Hb and intracellular Mb, would desaturate during exercise with a similar time course.[56] Therefore, a greater contribution of Mb than Hb to the NIRS [deoxy(Hb+Mb)] signal would not invalidate the interpretation that changes in this signal reflect fractional O_2 extraction, the dynamic balance between $\dot{V}O_{2m}$ and $\dot{Q}O_{2m}$ in the volume of tissue under consideration.[56] This would apply even if a separation between the Hb and the Mb signal is not feasible or is not carried out. In the next sections of the manuscript, we will discuss why, in our opinion, fractional O_2 extraction is by itself of interest.

In terms of the intracellular signals detectable by NIRS, cytochrome oxidase can also absorb NIR light. However, there is substantial agreement that assessment of the redox state of mitochondrial cytochrome oxidase cannot be done in muscle because of Mb interference,[60] whereas it is possible in the brain cortex.[61]

3.3 *Which Variable Should be Considered?*

Upon the premise that NIRS oxygenation signals reflect fractional O_2 extraction (see below) in the investigated volume of tissue, the following question can be asked: is there a correlation between NIRS-derived oxygenation signals and PO_2 or Hb saturation in venous blood draining from the exercising muscle(s)? The answer to this question may not be straightforward, and conflicting results have been reported in the past. The issue is complicated by the problem of obtaining, particularly in humans, adequate samples of venous blood coming directly from the exercising muscle(s), without a "contamination" from blood coming from other muscles and/or other tissues.

Wilson et al.[62] found an excellent correlation between a NIRS oxygenation index and O_2 saturation determined in the venous blood draining from a dog *gracilis* muscle preparation. The data were subsequently confirmed in humans during forearm exercise.[53] On the other hand, in two studies[63,64] performed during constant work rate exercise on a cycle ergometer, the following phenomena were observed (in normoxia): a transient decrease in oxygenation following the transition to exercise, which was paralleled by a decrease in femoral vein O_2 saturation; these decreases were followed by a paradoxical muscle "reoxygenation," which was not associated with an increased O_2 saturation in the femoral vein. The latter, as expected, remained low for the remaining portion of exercise. In other words, following exercise onset an association between the two variables (decreased muscle oxygenation, decreased femoral vein O_2 saturation) was observed, whereas dissociation occurred during the remaining portion of the constant work rate exercise. A similar muscle oxygenation pattern has been observed in different muscles[14,65,66] for the oxygenated-Hb and -Mb ([oxy(Hb+Mb)]) signal (see below) while not for [deoxy(Hb+Mb)], which increased and then stayed constantly elevated during the remaining portion of the exercise.

Grassi et al.[65] reasoned that the muscle reoxygenation suggested by the [oxy(Hb+Mb)] time-course could derive from an increased blood flow to the skin, occurring for thermoregulatory purposes, as also suggested by the results obtained by Maehara et al.,[67] Chuang et al.,[68] and Davis et al.[69] An increased skin blood flow for heat dispersion would increase the [oxy(Hb+Mb)] signal, whereas would not substantially affect [deoxy(Hb+Mb)], since the enhanced blood flow would not be associated with an increased gas exchange. This was substantially confirmed in a study carried out by Koga et al.[37] These authors observed by continuous-wave NIRS (CW-NIRS) (see below and Ferrari et al.[24] for a definition), during whole body heating, a more pronounced increase of [oxy(Hb+Mb)] compared to that of [deoxy(Hb+Mb)]. The apparent increased oxygenation described by CW-NIRS was not observed when utilizing TD-NIRS[37] (see below). The confounding effects of an increased skin blood flow on oxygenation variables [in particular [oxy(Hb+Mb)] determined by CW-NIRS] have been confirmed in a study carried out by Messere and Roatta.[70]

These authors observed that most of the warming-induced increase of the sum between oxy- and deoxy-Hb and -Mb ([total(Hb+Mb)]) resulted from an increase in [oxy(Hb+Mb)], with a relatively small contribution by [deoxy(Hb+Mb)]. They also observed that the "contamination" of the muscle NIRS signal by an increased skin blood flow was substantially reduced by using spatially resolved (SRS) NIRS (see below). No increases in muscle O_2 saturation (SO_{2m}, see below) were observed, by utilizing SRS-NIRS, in the *vastus lateralis* of subjects performing dynamic knee-extension exercise at 20% of MVC after thigh heating at 37°C and 42°C.[71] The unchanged SO_{2m} was observed in the presence of a marked increase in cutaneous vascular conductance.[54]

Several studies have confirmed the presence of a good or very good correlation between the [deoxy(Hb+Mb)] signal, or other tissue oxygenation variables determined by NIRS, and venous O_2 saturation, both in an animal model of isolated muscle *in situ*[14,72,73] and in exercising humans.[20,30,74,75] In Wüst et al.,[72] the correlation was worse during the initial part of contraction period, which in that model is associated with a forceful "muscle pump" effect and a substantial blood squeezing resulting from the sudden onset of tetanic contractions.

The first NIRS instruments widely utilized on skeletal muscle adopted, as a deoxygenation index, the differential absorption of light between 760 and 800 (or 850) nm.[52,53,67,76–83] The approach was based on the absorption characteristics of NIR light by the chromophores of interest at the two wavelengths. Subsequent instruments allowed the obtainment of relative (with respect to an initial value arbitrarily set equal to zero) or absolute [oxy(Hb+Mb)] and [deoxy(Hb+Mb)] values. The sum of [oxy(Hb+Mb)] and [deoxy(Hb+Mb)] indicates the total Hb + Mb volume ([total(Hb+Mb)]) in the tissue under consideration. Since total [Mb] cannot change acutely during exercise, changes in [total(Hb+Mb)] would reflect a vasodilation or an increased capillary hematocrit in the tissue under consideration. Absolute values of the above-mentioned variables can be obtained by the more technologically sophisticated (and more expensive) SRS, TD, or frequency-domain (FD) instruments, whereas the less technologically sophisticated, less expensive, and more widely utilized CW instruments allow only relative values (for a more detailed discussion on this topic, see below and Ferrari et al.[18,21,24]). This represents a limitation, which can be at least mitigated by performing a "physiological calibration" at the end of each test, by a transient ischemia of the investigated limb, obtained by applying for a few minutes a markedly suprasystolic pressure by a cuff, "upstream" of the region of investigation (see, e.g., Fig. 1 in Porcelli et al.[84]). This maneuver is not too uncomfortable for the subject. The range of values of [deoxy(Hb+Mb)] obtained in the muscle between rest and the condition of full extraction (i.e., when the [deoxy(Hb+Mb)] signal reaches a "plateau" after 4 to 5 min of ischemia) should be related to the range of $C(a\text{-}v)O_{2m}$ values between rest and full extraction. By adopting this physiological calibration, "semiquantitative" fractional O_2 extraction values can be obtained, also by CW-NIRS instruments.

Some controversy has been raised[85,86] about which one of the two variables ([oxy(Hb+Mb)] or [deoxy(Hb+Mb)]) should more precisely reflect changes in skeletal muscle fractional O_2 extraction. As discussed by Grassi,[87] the time-course of [deoxy(Hb+Mb)] (while not that of [oxy(Hb+Mb)]) during a constant work rate exercise[65] qualitatively reflects, rather closely, the time courses of variables related to fractional O_2 extraction across a wide spectrum of experimental models, such as $C(a\text{-}v)O_2$ determined across a contracting isolated *in situ* muscle preparation[88] or an exercising limb,[11,20] microvascular PO_2 (PO_{2mv}) determined by phosphorescence quenching in rat *spinotrapezius* muscle,[89] intracellular PO_2 determined by phosphorescence quenching in isolated amphibian muscle fiber.[90] Similar kinetics between PO_{2mv} and [deoxy(Hb+Mb)] during electrically stimulated contractions in the rat *gastrocnemius* were observed.[91] A study described a very similar pattern, also, for deoxy-Mb determined by ^{1}H-MRS[17]. In other words, during constant work rate exercise (or isometric contractions of constant metabolic rate), a series of variables related to O_2 extraction, spanning from $C(a\text{-}v)O_2$ across a whole limb to Mb saturation, show a very similar time-course: unchanged versus rest for a few seconds (suggesting an adequacy of $\dot{Q}O_{2m}$ with respect to $\dot{V}O_{2m}$—this information is relevant in terms of the factors determining the $\dot{V}O_2$ kinetics[3,4,10]), then a rapid exponential increase (the increase $\dot{V}O_{2m}$ exceeds the increase in $\dot{Q}O_{2m}$) to a new steady state, in which the ratio $\dot{V}O_{2m}/\dot{Q}O_{2m}$ reaches a new equilibrium, although at a higher value compared to that at rest. In other words, [deoxy(Hb+Mb)] (while not [oxy(Hb+Mb)]) belongs to this family of variables related to fractional O_2 extraction. This indirectly but strongly supports the role of [deoxy(Hb+Mb)] as an estimate of skeletal muscle fractional O_2 extraction. The concept is further strengthened by the issue of [oxy(Hb+Mb)] increases deriving from the enhanced blood flow to the skin for thermoregulatory purposes, as discussed above.

Strictly speaking, an increased [deoxy(Hb+Mb)] can be considered an estimate of an increased fractional O_2 extraction only if [total(Hb+Mb)] is constant, which is not always the case in exercising skeletal muscles. However, there is ample evidence[14,18,65,86,92–94] suggesting that changes in [deoxy(Hb+Mb)] are less influenced by changes in [total(Hb+Mb)] compared to changes in [oxy(Hb+Mb)]. Adami et al.[92] observed a good correlation between the increase in [deoxy(Hb+Mb)] and the decrease in femoral blood flow during passive head-up tilting, in the presence of a presumably unchanged $\dot{V}O_{2m}$. The data confirm the role of changes in [deoxy(Hb+Mb)] in evaluating O_2 extraction in the presence of acute and passively induced changes in $\dot{Q}O_{2m}$. In the study, passive head-up tilt induced also an increased venous blood volume, which

complicated the interpretation of [deoxy(Hb+Mb)] as an index of O_2 extraction;[92] venous blood pooling is, however, unlikely to occur during exercise. In any case, the issue of the influence of blood volume has been substantially overcome by a recent study[94] in which the authors calculated, on the basis of the assumption that during an arterial occlusion changes in [oxy(Hb+Mb)] and [deoxy(Hb+Mb)] should occur with a 1:1 ratio, a useful "blood volume correction factor," which can be applied to both [oxy(Hb+Mb)] and [deoxy(Hb+Mb)].

As mentioned above, SRS-, TD-, or FD-NIRS instruments allow the measurement of absolute values of [oxy(Hb+Mb)] and [deoxy(Hb+Mb)].[24] By utilizing these values, a tissue oxygenation index (TOI) can be calculated, as the ratio [oxy(Hb+Mb)]/[total(Hb+Mb)].[95] As an oxygenation variable, TOI is appealing since it is expressed as a percentage, similarly to arterial O_2 saturation (SO_{2a}). Since it includes [oxy(Hb+Mb)], however, also TOI may be more significantly influenced by skin blood flow compared to [deoxy(Hb+Mb)].

3.4 *Physiological Variables of Functional Evaluation*

Which of the variables in the Fick equation [Eq. (1)] can be at least estimated by NIRS? The answer is: all of them, although in order to determine $\dot{V}O_{2m}$ or $\dot{Q}_m$ some specific (and rather unphysiological) or invasive protocols need to be adopted. When these protocols are not utilized, the oxygenation indices obtained by NIRS evaluate the dynamic balance between $\dot{V}O_{2m}$ and $\dot{Q}O_{2m}$. In other words, these indices allow the estimation of skeletal muscle fractional O_2 extraction, a proxy of $C(a\text{-}v)O_{2m}$ (see also below).

3.4.1 *Skeletal muscle (or regional) blood flow*

De Blasi et al.[96] determined forearm $\dot{Q}$ by NIRS, at rest and after hand exercise, by inducing a 50-mmHg venous occlusion by a cuff. They reasoned that if venous outflow from a muscle is impeded by the cuff, whereas arterial inflow is not (or at least is only partially impeded), the increase in [total(Hb+Mb)] as a function of time should be related to $\dot{Q}$. The authors observed indeed an excellent correlation between forearm $\dot{Q}$, determined by NIRS as described above, and the increase in forearm volume determined by plethysmography. A similar approach was adopted by van Beekvelt et al.[32] No comparison with other methods to determine $\dot{Q}_m$ was made. Limitations of the approach relate to the fact that pressure by the cuff inevitably limits, although only in part, also arterial inflow, as recently confirmed.[97] Moreover, regional $\dot{Q}$ measurements could be carried out only during the recovery from exercise, and it is well known that the variable may rapidly change in the recovery phase.[6]

$\dot{Q}_m$ in the exercising calf muscles has been determined in humans by utilizing NIRS in association with the intravenous infusion of the NIR tracer indocyanine green (ICG).[98] ICG is a fluorescent dye approved by the U.S. Food and Drug Administration and European Medicines Agency for human use. Arterial blood was withdrawn by a pump and [ICG] was detected by photodensitometry, whereas NIRS optodes positioned over the muscle detected ICG transit in the microcirculation at several wavelengths. $\dot{Q}_m$ measurements were compared with those obtained by established methods. The main drawback of this elegant approach resides in its invasiveness, related to the need of obtaining arterial blood samples. The method has been utilized to determine $\dot{Q}_m$ in respiratory[99] and skeletal muscles[100]. Vogiatzis et al.[30] utilized it to evaluate the intramuscular matching between $\dot{V}O_{2m}$ and $\dot{Q}O_{2m}$.

3.4.2 *Skeletal muscle O_2 consumption*

Hamaoka et al.[101] determined by NIRS the initial rate of Hb and Mb deoxygenation in finger flexors of humans during ischemia performed at rest and immediately after submaximal exercise of different intensities, and considered this variable a reflection of $\dot{V}O_{2m}$. These authors observed a significant correlation between this rate and the concentrations of intramuscular metabolites (PCr and adenosine diphosphate), determined by ^{31}P-MRS, considered to be regulators of oxidative phosphorylation. They concluded that NIRS can quantify the rate of oxidative metabolism at rest and after exercise. The validity of the approach was subsequently confirmed.[102] The limitations are represented by the fact that the NIRS measurements need to be carried out in ischemic conditions and that the measurements are possible only in the recovery phase following exercise, during which $\dot{V}O_2$ rapidly decreases.[6]

While ischemic contractions do not represent a physiological condition, a "less unphysiological" experimental model would be one in which blood flow is held constant. Also in this case, according to the rearranged Fick equation [Eq. (2)], and after considering [deoxy(Hb+Mb)] a proxy of $C(a\text{-}v)O_{2m}$, the increase in [deoxy(Hb+Mb)] should represent an estimate of $\dot{V}O_{2m}$. This has indeed been experimentally confirmed in the study by Wüst et al.,[72] which carried out in an isolated dog *gastrocnemius in situ* preparation. In the study, $\dot{Q}_m$ was held constant by a pump, $\dot{V}O_{2m}$ was calculated by the Fick equation across the muscle and NIRS optodes were directly applied on the surface of the exposed muscle. During metabolic transitions, the kinetics of adjustment of [deoxy(Hb+Mb)] was very similar to the kinetics of adjustment of $\dot{V}O_{2m}$.

Brief sequential ischemic periods carried out during the recovery from exercise/contractions have been utilized in order to determine, by NIRS, the kinetics of recovery of $\dot{V}O_{2m}$ following exercise.[94,103–106] Also in this case, the rate of increase in [deoxy(Hb+Mb)] (and/or the rate of decrease in [oxy(Hb+Mb)]) during each of the brief ischemic periods is considered an estimate of $\dot{V}O_{2m}$. The kinetics of recovery of $\dot{V}O_{2m}$, as determined by the abovementioned protocol, has been shown to be closely correlated with two well-established variables of functional evaluation of skeletal muscle oxidative metabolism (frequently and incorrectly termed "oxidative capacity" by some of these authors): (1) the kinetics of recovery of [PCr], as determined by ^{31}P-MRS following exercise[104] and (2) maximal adenosine diphosphate-stimulated mitochondrial respiration, as determined by high-resolution respirometry in isolated and permeabilized skeletal muscle fibers.[106]

3.4.3 *Skeletal muscle fractional O_2 extraction*

As discussed above, in "normal" experimental conditions, oxygenation variables obtained by NIRS allow the evaluation of the dynamic balance between $\dot{V}O_{2m}$ and $\dot{Q}O_{2m}$, in other words of skeletal muscle fractional O_2 extraction, a proxy of $C(a\text{-}v)O_{2m}$. NIRS oxygenation variables can represent only a proxy of $C(a\text{-}v)O_{2m}$ since they are usually expressed in relative terms, and the proportional contributions of arterial and venous blood to the overall signal are unknown. In the next paragraphs, we will provide examples of conditions in which analysis of

skeletal muscle fractional O_2 extraction appears relevant and of physiological interest.

The capacity for prolonged exercise depends upon the capacity to supply the exercising muscles with O_2 in order to satisfy the metabolic requirements. During exercise, however, fractional O_2 extraction increases. In other words, compared to rest, the muscles (as other organs) utilize a greater percentage of the O_2 they receive by the cardiovascular system. We will discuss, later in this section, how this increase in fractional O_2 extraction could impair peripheral O_2 diffusion and oxidative metabolism through the resulting decrease in microvascular PO_2 (PO_{2mv}). Textbook physiology says that at the systemic level, in humans at rest, fractional O_2 extraction is ~25%, whereas during maximal exercise, the variable increases to ~75%. In steady-state conditions during exercise, the relationship between $\dot{Q}$ and $\dot{V}O_{2p}$ is linear, with a slope of 5 (1 L min^{-1} increase in $\dot{V}O_{2p}$ is accompanied by a 5 L min^{-1} increase in $\dot{Q}$). Considering that ~1/5 of the volume of arterial blood is represented by O_2 ($CaO_2 \sim 200$ mL L^{-1} of blood), the linear relationship between $\dot{Q}O_2$ and $\dot{V}O_{2p}$ has a slope of 1. As a consequence of the linear $\dot{Q}$ (or $\dot{Q}O_2$) versus $\dot{V}O_{2p}$ relationship, systemic $C(a\text{-}v)O_2$ would increase hyperbolically as a function of work rate. This has indeed been observed during ramp incremental cycling exercise.[107]

The relationship between $\dot{Q}_mO_2$ and $\dot{V}O_{2m}$ is somehow different when determined at the microvascular level, which is the domain investigated by NIRS. During an incremental or ramp exercise, the NIRS-derived [deoxy(Hb+Mb)] follows a sigmoid pattern,[62,84,107–113] with a shallow increase at low work rates (suggesting a tighter matching between muscle $\dot{V}O_{2m}$ and $\dot{Q}O_{2m}$, presumably associated with the recruitment of more oxidative fibers), a linear intermediate portion (suggesting greater O_2 extraction and a progressively higher $\dot{V}O_{2m}/\dot{Q}O_{2m}$, presumably associated with the recruitment of less oxidative fibers), followed by a *plateau* at work rates approaching ~80% of $\dot{V}O_{2m,max}$.

At submaximal work rates, before the "plateau" is reached, the observation of higher fractional O_2 extraction for the same $\dot{V}O_{2m}$ (or work rate), in other words, a left-shifted [deoxy(Hb+Mb)] versus work rate curve would suggest an impaired $\dot{Q}O_{2m}$. On the other hand, a lower fractional O_2 extraction (right-shifted [deoxy(Hb+Mb)] versus work rate) would suggest an excess $\dot{Q}O_{2m}$ with respect to $\dot{V}O_{2m}$. This information may have profound physiological and pathophysiological significance (see below). The sigmoid shape of the [deoxy(Hb+Mb)] versus work rate curve has been shown to vary in muscles characterized by different fiber type compositions[49] and has been confirmed (with minor differences) also during supine exercise[114] as well as during incremental exercise in acute hypoxia.[115] A right-shifted [deoxy(Hb+Mb)] versus work rate curve was described in trained cyclists.[116]

The [deoxy(Hb+Mb)] kinetics during constant work rate exercise has been utilized to evaluate the effects on the rate of adjustment of oxidative metabolism by a "prior" or "warm up" or "increased baseline" exercise,[72,117–119] or to investigate the effects on microvascular fractional O_2 extraction by interventions such as dietary nitrate supplementation, aimed at increasing microvascular nitric oxide (NO) availability.[120] For example, the increased muscle oxygenation observed by Gurd et al.[118] following a prior heavy intensity exercise suggests an enhanced O_2 availability, which should have contributed to the observed faster pulmonary $\dot{V}O_2$ kinetics. In other studies, the results were not conclusive. Bailey et al.,[117] for example, observed after oral nitrate supplementation, a decreased muscle O_2 extraction, which, however, could not be simply attributed to enhanced O_2 delivery since it was associated with a decreased $\dot{V}O_2$.

Skeletal muscle fractional O_2 extraction determined by NIRS can also give valuable physiological and pathophysiological insights into the integrated responses to exercise (and to other conditions) involving different muscle groups or skeletal muscles and other organs, as well as into the physiological mechanisms of $\dot{Q}_m$ regulation.

For example, arm $\dot{Q}$ (by thermodilution) and muscle oxygenation (by NIRS) were measured at the transition from arm to arm + leg exercise.[66] During maximal exercise, arm $\dot{Q}$ decreased at the transition, to a degree that $\dot{V}O_{2m,max}$ and muscle oxygenation were compromised. Thus, NIRS can give valuable insights into the coordination of $\dot{Q}_m$ and $\dot{V}O_{2m}$ adjustments to exercise between different muscle groups, in conditions of increased O_2 demands and/or limited $\dot{Q}O_{2m}$ (see also below).

Turner et al.[121] observed [deoxy(Hb+Mb)] increases both in limb locomotor muscles and in respiratory muscles after interventions, such as inspiratory muscle loading performed during cycling exercise at 80% of $\dot{V}O_{2p,max}$, or after increasing work rate from 80% to 100% of $\dot{V}O_{2p,max}$. The results suggest a limited $\dot{Q}O_{2m}$, both in locomotor and in respiratory muscles, which may impair exercise tolerance during maximal exercise or in conditions of increased respiratory muscles work.

By applying lower body negative pressure, Soller et al.[122] induced a progressive decrease of central blood volume and a decreased stroke volume, which were paralleled by a decreased muscle oxygenation from vasoconstriction. According to these authors, skeletal muscle deoxygenation determined by NIRS could be utilized as an early indicator of central hypovolemia and impending cardiovascular collapse.

NIRS has also been utilized to elucidate basic physiological mechanisms of $\dot{Q}_m$ regulation, such as the metabolic modulation of sympathetic vasoconstriction (functional sympatholysis) by exercise[123] or by tissue hypoxia.[124] By simultaneously measuring sympathetic nerve activity by microelectrodes and forearm oxygenation by NIRS, Hansen et al.[123] provided evidence in favor of functional sympatholysis. Reflex sympathetic activation, which consistently decreased oxygenation in resting forearm muscle, had no effect on oxygenation when the muscles were exercised.

The [deoxy(Hb+Mb)] plateau during an incremental exercise can be considered an estimate of the maximal capacity of O_2 extraction during exercise and has been utilized in several studies[84,109–113,125–127] as a variable of functional evaluation of skeletal muscle oxidative metabolism.

Venous blood draining from exercising muscles still contains small but significant amounts of O_2; in other words, fractional O_2 extraction is not 100%.[128–130] Which are the normal values of fractional O_2 extraction across skeletal muscle during maximal exercise? According to Knight et al.,[128] during cycling, the value can go up to ~90%, as determined by invasive measurements carried out across an exercising limb. A fractional O_2 extraction value of ~85%, also in this case determined invasively across an exercising limb, was observed during maximal knee-extensor exercise,[130] which is an exercise paradigm (involvement of a relatively low small muscle mass) in which cardiovascular constraints to O_2 delivery are not present or are significantly reduced, and skeletal muscle blood flow can be three times higher than during conventional cycle ergometer exercise.

These values are very similar to those obtained in normal subjects by NIRS for [deoxy(Hb+Mb)] at peak exercise, expressed as a percentage of the maximal [deoxy(Hb+Mb)] value observed during a transient limb ischemia.[84]

Although, in normal subjects, $\dot{V}O_{2p,max}$ is traditionally considered to be mainly limited by maximal cardiac output ($\dot{Q}_{max}$) and maximal cardiovascular O_2 delivery ($\dot{Q}O_{2\,max}$),[131] the role of peripheral factors cannot be overlooked.[132,133] If one considers the Fick equation [see Eq. (1)] applied to conditions of maximal exercise, it is obvious that the main difference between subjects with high $\dot{V}O_{2p,max}$ and subjects with low $\dot{V}O_{2p,max}$ resides in $\dot{Q}O_{2\,max}$, and not in $C(a\text{-}v)O_{2\,max}$. However, the concept that $\dot{Q}O_{2\,max}$ represents cardiovascular limitations and $C(a\text{-}v)O_{2\,max}$ or maximal fractional O_2 extraction represent peripheral factors is an oversimplification.[134] In an in-series O_2 transport system, every step must play a role in affecting the overall outcome, and the different functions are strictly interconnected.[133] $\dot{Q}O_2$, by setting microvascular PO_2 (PO_{2mv}), determines peripheral O_2 diffusion and therefore fractional O_2 extraction. In the presence of an elevated $\dot{Q}O_2$, as it occurs in athletes or after training, peripheral O_2 diffusing capacity (DO_{2m}) must increase in order to allow a large fractional O_2 extraction.

Although the issue is debated,[135] in skeletal muscle, an O_2 extraction limit is present, which contributes to $\dot{V}O_{2p,max}$ and $\dot{V}O_{2m,max}$ limitation; moreover, a limitation in peripheral O_2 diffusion appears to be the major factor responsible for the limited extraction.[133] Exercise training may increase muscle fractional O_2 extraction by elevating muscle(s) DO_{2m} to a greater extent than $\dot{Q}O_{2m,max}$.[35,42] Kalliokowski et al.[136] observed by positron emission tomography that fractional O_2 extraction during exercise is higher in endurance trained athletes compared to untrained controls. According to Whipp and Ward,[137] systemic $C(a\text{-}v)O_{2\,max}$ increases hyperbolically with $\dot{V}O_{2p,max}$. In other words, also in healthy subjects, fractional O_2 extraction is relevant in determining $\dot{V}O_{2p,max}$. This concept applies even more strongly to patients or to conditions in which oxidative metabolism is impaired (see below).

The "scenario" depicted above can be looked at also from another perspective: an increased O_2 extraction may impair peripheral O_2 diffusion. This could occur also during submaximal exercise. A fall in oxygenation (corresponding to an increased $\dot{V}O_{2m}/\dot{Q}O_{2m}$) suggests a less pronounced or inadequate vascular response in relation to an increased $\dot{V}O_{2m}$. This would determine a decreased PO_{2mv} and mean capillary PO_2, a decreased driving pressure for peripheral O_2 diffusion from capillaries to mitochondria,[42,43,138] and a functional impairment of skeletal muscle oxidative metabolism.

The phenomenon could also occur transiently in the early phase of a constant work rate exercise. This is suggested by the transient "overshoot" of skeletal muscle fractional O_2 extraction (usually determined by analyzing the [deoxy(Hb+Mb)] time-course), which has been described in contracting *in situ* dog muscle[14] as well as during constant work rate exercise in different populations (see also below), such as patients with chronic heart failure (CHF),[95,139] patients with metabolic myopathies,[140] type 2 diabetes,[141] chronic obstructive pulmonary disease,[142] healthy subjects undergoing bed rest deconditioning[84] or bed rest associated with hypoxia,[127] healthy subjects during arm exercise.[81] In the latter study, the overshoot in skeletal muscle fractional O_2 extraction, determined by NIRS, was closely associated with an overshoot in $C(a\text{-}v)O_2$, determined invasively across the exercising muscles. Goodwin et al.[14] experimentally slowed $\dot{Q}O_{2m}$ kinetics in isolated *in situ* dog muscle and observed an enhanced [deoxy(Hb+Mb)] overshoot and a slowed $\dot{V}O_{2m}$ kinetics. In human subjects acutely exposed to normobaric hypoxia, Bowen et al.[33] observed a slower $\dot{V}O_{2p}$ kinetics and a more pronounced [deoxy(Hb+Mb)] transitory increase, although the magnitude of the overshoot was unaffected. The O_2 extraction overshoot, occurring early during a constant work rate exercise, is considered to be caused by a transient mismatch between $\dot{Q}O_{2m}$ and $\dot{V}O_{2m}$.[42,43,138,143,144] The mismatch could derive from a suboptimal NO signaling within the muscle, which could "uncouple," both temporally and spatially, the heterogeneous microvascular $\dot{Q}O_{2m}$ increase from the presumably heterogeneous $\dot{V}O_{2m}$ increase.[43,138]

The transiently increased fractional O_2 extraction is associated with a transiently decreased PO_{2mv}.[42,43,138,144] This phenomenon has been described in animal models, in which PO_{2mv} was determined by phosphorescence quenching.[35,145] As mentioned above, the decreased PO_{2mv} would reduce the driving pressure for peripheral O_2 diffusion and impair oxidative metabolism. The impairment would be functionally significant also because it would be temporally located in the critical early phase following an increase in metabolic intensity.[3,4,6,10]

A partially different approach to the [deoxy(Hb+Mb)] overshoot has been taken by Murias et al.[146] These authors utilized a "normalized" $[\text{deoxy}(\text{Hb}+\text{Mb})]/\dot{V}O_{2p}$ ratio, in which both variables are expressed as a percentage of the response. In order to calculate the ratio, the $\dot{V}O_{2p}$ signal is left-shifted by 20 s, which is assumed to represent, on average, the delay between the $\dot{V}O_{2m}$ increase (the variable of interest in the comparison with [deoxy(Hb+Mb)]) and the $\dot{V}O_{2p}$ increase,[3,4] which is the variable usually determined. Another assumption related to this approach is that $\dot{V}O_{2p}$ kinetics equals $\dot{V}O_{2m}$ kinetics. This is not exactly true (see the discussion in Koga et al.[42]): the two variables have similar kinetics[11] but with a substantial intersubject variability. Moreover, [deoxy(Hb+Mb)] and $\dot{V}O_{2p}$ increases are, in relative terms, substantially different, and therefore, the normalization (percentages of responses) likely introduces a distortion in the calculation of the ratio.

In another approach, Ferreira et al.[143] assumed (as Murias et al.[146] did) that $\dot{V}O_{2p}$ kinetics equals $\dot{V}O_{2m}$ kinetics, and that [deoxy(Hb+Mb)], as an estimate of O_2 extraction, quantitatively reflects $C(a\text{-}v)O_2$; the authors then calculated microvascular blood flow adjustment by solving the Fick equation. The main limitations of this approach, in our opinion, reside in the assumptions: the first one ($\dot{V}O_{2m}$ kinetics equals to $\dot{V}O_{2p}$ kinetics) has been discussed above, but also the second one ([deoxy(Hb+Mb)] as a quantitative measurement of $C(a\text{-}v)O_2$) is not strictly correct: even when convincing "physiological calibration" methods are utilized, [deoxy(Hb+Mb)] allows at best only a semiquantitative estimation of $C(a\text{-}v)O_2$, and therefore, its use in replacing $C(a\text{-}v)O_2$ in mathematical equations seems questionable.[147] Moreover, the approach by Ferreira et al.[143] assumes that the contribution by Mb to the NIRS signals is zero; as discussed above, this is likely not the case.

During the recovery following exercise, the decrease in $\dot{V}O_{2p}$ and $\dot{V}O_{2m}$ follows an exponential time course, even in the presence of a sudden decrease in mechanical power output.[6] In these conditions, the "excess $\dot{V}O_{2m}$," with respect to the needs of myosin ATPase, derives essentially from the resynthesis of PCr, which occurs by oxidative phosphorylation. Upon the assumption of equilibrium of the CK reaction, the recovery rate of [PCr] following exercise is therefore a function of

mitochondrial ATP production. The rate of recovery of [PCr] following exercise has often been utilized as a tool of evaluation of skeletal muscle oxidative metabolism.[15,104] Another variable frequently utilized as a functional evaluation tool is the NIRS-determined reoxygenation kinetics during the recovery. This kinetics is a function of the interplay between the kinetics of $\dot{Q}O_{2m}$ and the kinetics of $\dot{V}O_{2m}$, and cannot discriminate between the two. However, a good agreement was found between the rate of recovery of [PCr] (as determined by ^{31}P-MRS) and the reoxygenation kinetics (as determined by NIRS) following moderate-intensity exercise that is the absence of significant drops in pH.[80] These authors concluded that the reoxygenation kinetics during the recovery from a moderate-intensity exercise could represent a functional evaluation tool of oxidative performance.

3.4.4 *Other variables and purposes*

Several studies have been conducted with the aim to identify, by NIRS, variables of functional evaluation of oxidative metabolism (thresholds), such as the ventilatory threshold,[148,149] the "EMG threshold,"[150] the onset of blood lactate accumulation during incremental exercise,[151] or the "maximal lactate steady state."[152] Most of these studies observed a nice correlation between NIRS-obtained indices and more traditional variables utilized to identify the various thresholds.[148,149,151,152]

Broxterman et al.[153] utilized NIRS, together with EMG and the analysis of the curvature constant of the power versus time to exhaustion relationship, in order to evaluate the role of central and peripheral fatigue during exercise with small muscle mass (handgrip) in conditions of blood flow occlusion. Central (brain) factors were identified as responsible for fatigue during submaximal elbow flexors exercise in severe hypoxia;[154] in the study, the authors utilized muscle and brain NIRS, evoked and EMG responses to electrical muscle stimulation, transcranial magnetic stimulation, and motor-evoked potentials.

Soller et al.[122] developed a mathematical model, which correlated NIRS spectra with muscle pH (pH_m) values obtained invasively by sensors placed in the muscle. They could then predict pH_m values on the basis of NIRS spectra, and determined, during incremental exercise, an "H^+ threshold," which correlated with classic "metabolic thresholds".

Towse et al.[155] utilized NIRS blood volume and muscle oxygenation changes to confirm the blood-oxygenation-level-dependent (BOLD) mechanism underlying the changes of magnetic resonance imaging signal intensity observed following brief, single contractions.

Several NIRS studies have been performed in electrically stimulated contractions. For instance, McNeil et al.[156] observed by NIRS a greater metabolic demand, associated with greater fatigue during electrically stimulated (versus voluntary), contractions carried out at the same torque production. On the contrary, the local O_2 demand of the *biceps brachii* was found similar between electrical muscle stimulation and maximal voluntary isometric contractions.[157]

4 Aging, Immobilization, Hypoxia

4.1 *Aging*

By combining ^{31}P-MRS, Doppler ultrasound imaging, and NIRS Layec et al.[158] observed a higher ATP cost of contractions during plantar flexion exercise in elderly subjects (73-year old) versus young activity-matched controls, in association with higher $\dot{Q}_m$ and $\dot{Q}O_{2m}$ and an unchanged microvascular fractional O_2 extraction. These results point to a higher energy cost of contractions as the main factor responsible for the reduced exercise capacity in the elderly. At least in part, different results were described by Ferri et al.,[125] who observed in 78-year old subjects, higher skeletal muscle (*vastus lateralis*) fractional O_2 extraction, at the same absolute work rate, suggesting an impairment of $\dot{Q}O_{2m}$. This phenomenon was observed both during cycle ergometer exercise (in which the limitations to oxidative metabolism are mainly related to cardiovascular O_2 delivery) and during one-leg knee-extension exercise,[125] in which cardiovascular limitations are substantially reduced.[130]

$\dot{V}O_{2p}$ kinetics is slower in the elderly. Murias et al.[159] observed that this slower kinetics was associated with a larger overshoot in the $[\text{deoxy}(Hb + Mb)]/\dot{V}O_{2p}$ (both variables were expressed as a percentage of their total response, see also above) ratio, suggesting, according to the authors, an impaired adjustment of $\dot{Q}O_{2m}$. This would be in agreement with observations of a blunted vasodilatory dynamics in arterioles of old rats.[160] Exercise training speeded $\dot{V}O_{2p}$ kinetics and reduced the $[\text{deoxy}(Hb + Mb)]/\dot{V}O_{2p}$ overshoot,[159] suggesting an improved matching between $\dot{Q}O_{2m}$ and $\dot{V}O_{2m}$. In another study, no $[\text{deoxy}(Hb + Mb)]/\dot{V}O_{2p}$ overshoot was observed in chronically trained elderly subjects.[161] It should be considered, however, that in these studies,[159,161] the overshoot in [deoxy(Hb +Mb)] was only relative to $\dot{V}O_2$; in other words, no "real" [deoxy(Hb+Mb)] overshoot was present.

4.2 *Immobilization/Microgravity and Countermeasures*

Higher skeletal muscle fractional O_2 extraction for the same work rate, a reduced peak capacity of O_2 extraction during an incremental exercise, and an overshoot of [deoxy(Hb+Mb)] during constant work rate exercise were observed in healthy young subjects undergoing 35 days of bed rest.[84,111] These findings were observed during both cycle ergometer exercise[84] and one-leg knee extension exercise.[111] These results include skeletal muscle oxidative metabolism among the "victims" of profound inactivity. Since bed rest studies have been considered a "proxy" of microgravity (see the discussion in Porcelli et al.,[84] Salvadego et al.[111]), the same concept applies to microgravity. Superposition of normobaric hypoxia (equivalent to an altitude of about 4000 m) upon 10 days of bed rest attenuated, during one-leg knee extension exercise (but not during cycle ergometer exercise), the impairment of the peak capacity of fractional O_2 extraction and the magnitude of the overshoot, which were observed following bed rest alone.[127] An enhanced perfusive and diffusive O_2 delivery deriving from the hypoxia-induced increased [Hb] may explain, at least in part, this unexpected finding.

Training programs carried out during 21-day forearm immobilization by a plaster cast were found effective in preventing the decline in muscle oxidative function, endurance, and strength.[162]

4.3 *Hypoxia*

During acute moderate normobaric hypoxia (inspired O_2 partial pressure [PO_{2I}] 118 mmHg, equivalent to an altitude of ~2500 m), muscle deoxygenation during an incremental cycle exercise is not significantly different, for the same absolute work rate, compared to normoxic conditions.[163] The data

suggest higher $\dot{Q}_m$ in hypoxia compared to normoxia and, thus, the existence of control mechanisms, which effectively match $\dot{Q}O_{2m}$ to $\dot{V}O_2$. This effective matching is presumably lost at higher altitudes. Billaut et al.[164] collected muscle and brain oxygenation data to gain insights into the central and peripheral factors affecting muscle fatigue in conditions of limited O_2 delivery. They described a trend toward lower *vastus lateralis* muscle oxygenation in subjects performing repeated sprints and acutely exposed to a slightly more pronounced (compared to that of the previously mentioned study) moderate hypoxia ($FO_{2I} = 0.138$, PO_{2I} 98.4 mmHg). Subudhi et al.[165] described greater fractional O_2 extraction values for the same absolute work rate in subjects acutely exposed to hypobaric hypoxia (PO_{2I} 86 mmHg) corresponding to an altitude of ~4300 m. Interestingly, no differences versus acute hypoxia were described, in the same subjects, in chronic hypoxia (5-day acclimatization at 1830 m and 1-day at 4300 m). In all the mentioned studies,[163–165] marked differences between normoxia and acute or chronic hypoxia were described for prefrontal cortex oxygenation.

Unchanged muscle oxygenation during exercise was described after versus before a 72-day acclimatization to chronic hypobaric hypoxia (Mt. Everest expedition);[166] the unchanged muscle deoxygenation was associated with an increased hypoxic tolerance and with enhanced acute hypoxic ventilatory response and cerebral oxygenation. In another Mt. Everest expedition, a slower muscle (thenar eminence) reoxygenation kinetics was observed during vascular occlusion tests carried out at 4900 and 5600 m.[167]

A more pronounced muscle deoxygenation during constant work rate exercise was described during acute hypoxia,[67] obtained either by having the subjects breath air with a FO_{2I} of 0.15 or by carbon monoxide loading aimed at increasing carboxyhemoglobin saturation. Interestingly, the accelerated deoxygenation was described only during exercise above the "lactate threshold," that is, at an exercise intensity at which O_2 supply to the exercising muscles becomes critical.[2,3,4,10]

A greater deoxygenation of accessory respiratory muscles during voluntary isocapnic hyperpnea was observed in hypoxia ($FO_{2I} = 0.10 - 0.11$) versus normoxia,[168] in association with a higher muscle activation determined by EMG.

5 Patients Populations

In this section, we will present an overview of some representative studies in which the variables of functional evaluation discussed in the previous section have been determined in adult patients' populations. The aims were to evaluate the evolution of impairments as a function of time, to identify correlations with clinical symptoms, to evaluate the effects of therapeutic or rehabilitative interventions, and to gain pathophysiological and diagnostic insights.

A systematic review on muscle NIRS measurements in the clinical care of term and preterm neonates has been recently published.[169]

5.1 *Chronic Heart Failure*

Several studies have been conducted by utilizing NIRS in patients with CHF. The complex interplay between central and peripheral factors in determining the pathophysiology of the disease and the impaired exercise tolerance in CHF patients has been discussed in detail in the review by Poole et al.[138] A subsequent review by the same group[144] discussed, from the same perspective, the effects of training in this patient population.

Wilson et al.[62] observed higher fractional O_2 extraction, for the same absolute work rate, in CHF patients versus age-matched healthy controls; the results suggest an impaired $\dot{Q}O_{2m}$ in the patients, presumably a consequence of the impaired cardiac function. A similar pattern was observed by another study in CHF patients.[126] Interestingly, in this study, the impaired $\dot{Q}O_{2m}$ response was not affected by 3 months of light-to-moderate exercise training. The training intervention, on the other hand, increased the maximal capacity of skeletal muscle fractional O_2 extraction during exercise, allowing the patients to reach values not different from those of controls.

A very similar pattern to that described in CHF patients[126] before training was observed in patients evaluated after a successful heart transplantation,[109] carried out in the treatment of severe CHF. The data suggest that the surgical intervention, although improving cardiac function, did not significantly improve the $\dot{Q}O_{2m}$ impairment at submaximal exercise and the reduced capacity of maximal fractional O_2 extraction by skeletal muscles. Also in patients undergoing heart transplantation, a complex interaction between central and peripheral functions limits exercise tolerance.[109]

Sperandio et al.[139] described by NIRS in CHF patients, during constant work rate submaximal exercise, a transient overshoot in fractional O_2 extraction (increased [deoxy(Hb+Mb)]), presumably leading to a decreased PO_{2mv} and to an impaired peripheral O_2 diffusion during the critical early phase of the metabolic transition. This overshoot could derive from a suboptimal intramuscular NO signaling.[138] The [deoxy(Hb+Mb)] overshoot is qualitatively equivalent and has the same pathophysiological meaning with respect to the transient PO_{2mv} undershoot described in the *spinotrapezius* muscle of CHF rats.[170] In addition, Sperandio et al.[139] observed that in CHF patients an increased NO bioavailability, obtained by the administration of sildenafil, eliminated the [deoxy(Hb+Mb)] overshoot, determined a faster $\dot{V}O_{2p}$ kinetics, and improved exercise tolerance. Bowen et al.[95] observed that the deoxygenation overshoot in CHF patients was attenuated by a prior exercise (aimed at enhancing $\dot{Q}O_{2m}$), which determined also a faster $\dot{V}O_{2p}$ kinetics, confirming the link between the overshoot and an impaired oxidative metabolism. On the basis of the deoxygenation and $\dot{V}O_{2p}$ kinetics, the authors identified two subgroups of patients: the most severely limited ones were those with an impaired skeletal muscle oxidative metabolism.[95]

Belardinelli et al.[82] observed in CHF patients a slower reoxygenation kinetics following exercise, in association with a slower $\dot{V}O_{2p}$ kinetics. In another study, Hanada et al.[171] observed that the slower reoxygenation kinetics in CHF patients was associated with a markedly slower kinetics of recovery of [PCr], as determined by ^{31}P-MRS. As mentioned above, a slower reoxygenation kinetics during the recovery after exercise is considered an index of impaired skeletal muscle oxidative performance.[80] At least in part, different results were obtained by Kemp et al.:[172] according to these authors, in CHF patients the slowing of muscle reoxygenation kinetics was more pronounced than the slowing of [PCr] recovery; the data suggest a $\dot{Q}O_{2m}$ limitation.

A slower recovery kinetics of $\dot{V}O_{2m}$ (determined by NIRS by utilizing the method by Ryan et al.[94] mentioned above) following wrist-flexor exercise was observed in systolic CHF patients;[173] interestingly, whereas in control subjects, a 4-

week period of exercise training determined a faster recovery kinetics, this did not occur in the CHF patients. The authors concluded that the impairment of nonlocomotor muscles oxidative metabolism in the CHF patients was not reversible with a short period of exercise training. Moreover, the impairment was not simply due to deconditioning, but suggests a skeletal muscle dysfunction intrinsically associated with the disease, as also proposed in the reviews by Poole et al.[138] and Hirai et al.[144]

Mancini et al.[76] observed deoxygenation of accessory muscles of respiration (*serratus anterior*) in CHF patients (but not in controls) during submaximal and maximal cycle exercise; the observed deoxygenation may contribute to the exertional dyspnea typically described in these patients. It is well known that more severely affected CHF patients typically have a hyperventilatory response to exercise, which holds clinical and prognostic implications.[174] The hyperventilatory response, the decreased respiratory muscle strength, and the increased work of breathing would increase $\dot{Q}O_{2m}$ requirements of respiratory muscles, and could induce a "competition" for the limited available $\dot{Q}O_2$ between respiratory and locomotor muscles, contributing to the decreased exercise tolerance.[175] In support of this hypothesis, Borghi-Silva et al.[176] observed that respiratory muscles unloading, obtained in CHF patients by proportional assisted ventilation, increased exercise tolerance in association with an enhanced leg muscle oxygenation and blood volume determined by NIRS. These studies demonstrate how NIRS can give valuable pathophysiological insights into the interplay between different muscles in conditions of limited available $\dot{Q}O_2$.

A functional evaluation of skeletal muscle oxidative metabolism would be even more important in CHF patients with preserved ejection fraction, in whom peripheral impairments appear more significant than those usually observed in the more "traditional" CHF patients with reduced ejection fraction.[144,177] In this respect, very few data are available yet.

5.2 *Peripheral Arterial Disease and Diabetes*

During incremental exercise, PAD patients show near-maximal deoxygenation (with respect to the ischemic calibration) beginning from very low work rates.[79] In the same study, the reoxygenation kinetics following exercise was markedly slower in PAD patients; moreover, the half-time of this recovery was significantly correlated with the "ankle-arm index" (ratio of systolic blood pressures at the ankle and at the arm),[79] a clinical variable evaluating the severity of the disease. A markedly slower reoxygenation kinetics in PAD patients was confirmed in subsequent studies.[83,141]

In PAD patients, the involvement of the two legs is often markedly asymmetrical. Interestingly, McCully et al.[79] observed that the reoxygenation kinetics was markedly slower in the "bad" leg versus the "good" leg. According to McCully et al.,[83] analysis of reoxygenation kinetics by NIRS could be utilized as a screening tool for the presence of PAD.

In patients with PAD, NIRS has also been utilized to evaluate the effects of various therapeutic interventions. Whereas Beckitt et al.[178] described only modest improvements of muscle oxygenation during exercise in PAD patients treated with angioplasty, substantial positive effects were observed after the administration of sildenafil[179] or after an oral supplementation (beetroot juice) of nitrates.[180] Both these approaches aimed, although by exploiting different pathways, at increasing NO bioavailability in the microvasculature, thereby taking advantage of the vasodilatory effect of NO and also at improving the intramuscular matching between $\dot{V}O_{2m}$ and $\dot{Q}O_{2m}$.

What is the pathophysiological meaning of the slower reoxygenation kinetics in PAD patients? As a general rule, this kinetics is a function of the interplay between the kinetics of $\dot{Q}O_{2m}$ and the kinetics of $\dot{V}O_{2m}$ during the recovery following exercise. In PAD patients, the first variable is obviously impaired, but the second one could be impaired as well. This has been demonstrated by Bauer et al.,[141] who observed in PVD patients a slower recovery of reoxygenation also in the patients with reasonably normal calf blood flow responses, as evaluated by plethysmography.

The clinical relevance of NIRS applications in PAD has been very recently reviewed by Boezeman et al.,[181] who considered 183 manuscripts. These authors found sufficient evidence to use NIRS in the clinical setting for assessment of chronic compartment syndrome of lower extremities, and as a surveillance tool for the detection of "free flap failure". On the other hand, the clinical relevance of routine use of NIRS in other vascular diseases was found to be less clear.

For the same absolute work rate and $\dot{V}O_{2p}$, type 1 diabetic patients show (versus controls) higher [deoxy(Hb+Mb)], suggesting an impaired $\dot{Q}O_{2m}$, as also suggested by the lower $\dot{Q}_m$.[182]

An overshoot of [deoxy(Hb+Mb)] during constant work rate exercise has been described in patients with type 2 diabetes.[183] This observation was associated with slower $\dot{V}O_{2p}$ kinetics and appears compatible with an impaired $\dot{Q}O_{2m}$ adjustment, consequence of the microvascular impairment. The [deoxy(Hb+Mb)] overshoot described in diabetic patients is qualitatively equivalent and has the same pathophysiological meaning of the transient PO_{2mv} undershoot described in the exercising muscles of diabetic rats.[89]

In a review, Pedersen et al.[184] discussed the main methods to evaluate mitochondrial dysfunction in patients with PAD and type 2 diabetes. According to these authors, NIRS works better than ^{31}P-MRS in determining the severity of the impairment.

Manfredini et al.[185] utilized NIRS in order to find objective correlates between the onset of symptoms of claudication and the limited exercise tolerance in PAD patients, with and without diabetes. In order to do so, they determined the area under the [oxy(Hb+Mb)] or [deoxy(Hb+Mb)] curves (with respect to the baseline) during an incremental exercise. These areas evaluate (although in arbitrary units) the overall burden represented by the dynamic unbalance between $\dot{V}O_{2m}$ and $\dot{Q}O_{2m}$. The areas were greater in the PAD patients with diabetes and were correlated with the onset of symptoms. In the PAD patients with diabetes, however, ischemic symptoms were delayed and occurred at a level of deoxygenation which was significantly greater than in the patients with PAD alone, possibly as a consequence of a blunted pain perception and/or of a subjective higher tolerance for muscle discomfort associated with diabetes.

5.3 *Chronic Obstructive Pulmonary Disease*

By substituting nitrogen with helium in inspired air, gas density is reduced to $\sim 1/3$ of that of normal ambient air. After relieving the work of breathing by having COPD patients breathe a normoxic helium-O_2 mixture, Chiappa et al.[186] observed less leg deoxygenation by NIRS, faster $\dot{V}O_{2p}$ kinetics, and an increased exercise tolerance. The authors hypothesize that by decreasing the competition for the available $\dot{Q}O_2$ between respiratory and locomotor muscles, helium-O_2 breathing could increase leg $\dot{Q}O_{2m}$, thereby enhancing skeletal muscle oxidative metabolism.

This hypothesis was only in part confirmed in a subsequent study.[48] These authors determined by NIRS, by utilizing the ICG dye dilution method,[98] intercostal, and quadriceps muscles $\dot{Q}_m$ in COPD patients breathing room air or a normoxic helium-O_2 mixture. During cycling exercise at 75% of $\dot{V}O_{2p,max}$, helium-O_2 was associated with increases in $\dot{Q}_m$ in both intercostal and quadriceps muscles, together with an increased CaO_2 and with signs of enhanced exercise tolerance. The authors hypothesized that changes in lung mechanics induced by helium-O_2 likely increased stroke volume, $\dot{Q}$ and systemic $\dot{Q}O_2$. A subsequent study[187] went a step further. These authors subdivided COPD patients into dynamic "hyperinflators" and "nonhyperinflators," measured quadriceps $\dot{Q}_m$ by the ICG method[98] and calculated $\dot{Q}O_{2m}$. Helium-O_2 increased exercise tolerance and $\dot{Q}O_{2m}$ in both groups, but at least in part different mechanisms were involved: in hyperinflators, helium-O_2 increased CaO_2 and $\dot{Q}O_{2m}$ in the presence of no changes in $\dot{Q}$; in nonhyperinflators, helium-O_2 improved $\dot{Q}$, vascular conductance, and $\dot{Q}O_{2m}$. These studies nicely demonstrate that, also in COPD patients, NIRS can give valuable insights into the mechanisms regulating the integrated responses to conditions of increased O_2 demand.

Also, COPD patients showed the [deoxy(Hb+Mb)] overshoot during constant work rate exercise[142] and a slower reoxygenation kinetics during the recovery following exercise.[188,189] Following 6 weeks of high-intensity training, the reoxygenation kinetics became faster, in association with an increased citrate synthase activity and an increased exercise tolerance.[188]

5.4 *Metabolic Myopathies and Muscular Dystrophies*

Since NIRS oxygenation indices reflect skeletal muscle fractional O_2 extraction, they appear to be ideally suited to identify and evaluate the impairment of oxidative metabolism in patients with mitochondrial myopathies (MM) and myophosphorylase deficiency (McArdle disease, McA), in whom the incapacity to increase skeletal muscle fractional O_2 extraction represents a key pathophysiological mechanism (see the discussion in Grassi et al.[110]). Following the pioneering work by Bank and Chance,[78] Grassi et al.,[110] using NIRS, measured [deoxy(Hb+Mb)] at exhaustion ($[\text{deoxy}(\text{Hb}+\text{Mb})]_{\text{peak}}$) during an incremental exercise test to obtain the maximal capacity of skeletal muscle fractional O_2 extraction in MM and McA patients. The values were significantly lower than those of controls, and they were linearly correlated with $\dot{V}O_{2p,peak}$,[110] a close approximation of $\dot{V}O_{2p,max}$; they also were inversely related to the time-constant of $\dot{V}O_{2p}$ kinetics,[190] another classical variable of functional evaluation of skeletal muscle oxidative metabolism.[3,4,10] The most severely impaired patients could not increase their fractional O_2 extraction during exercise compared to the values obtained at rest, confirming observations obtained in previous studies by invasive measurements (see the discussion in Grassi et al.[110]). Moreover, $[\text{deoxy}(\text{Hb}+\text{Mb})]_{\text{peak}}$ values obtained in MM and McA patients were substantially lower than those obtained in patients with similar signs and symptoms but with a negative biopsy for any known metabolic myopathy.[110] In other words, NIRS allowed one to identify and quantify the metabolic impairment in MM and McA patients and could also offer diagnostic clues, for example, in the selection of patients on whom to perform a muscle biopsy.

Markedly lower [deoxy(Hb+Mb)] values at submaximal work rates were observed in MM and McA patients.[110,140] Lower [deoxy(Hb+Mb)] values at the same submaximal work rate, versus controls, were also observed in MM during incremental handgrip exercise.[191] These data suggest an excess in $\dot{Q}O_{2m}$ for the same $\dot{V}O_{2m}$ (and work rate), which represents one of the key pathophysiological mechanisms of the diseases.

MM and McA patients also showed a marked transient [deoxy(Hb+Mb)] overshoot during constant work rate exercise.[140] In McA, the overshoot disappeared in the presence of the "second wind" phenomenon, a sudden decrease in heart rate and perceived exertion occurring in these patients after a few minutes of exercise, or during a second exercise preceded by a few minutes by a prior exercise.[140] The second wind phenomenon is thought to derive from an enhanced sympathoadrenal response, which would allow the utilization of glucose deriving from blood or extramuscular sources and of free fatty acids, thereby bypassing the metabolic impairment (the inability to breakdown intramuscular glycogen) at the basis of the disease (see the discussion in Porcelli et al.[140]). In other words, in McA patients, NIRS confirmed the mechanism (sudden enhancement of oxidative metabolism) at the basis of the second wind phenomenon.

Another glycolytic defect leading to a decreased capacity to increase fractional O_2 extraction during exercise is muscle-type phosphofructokinase (M-PFK) deficiency. The impairment has been demonstrated by NIRS in humans[70] and in dogs.[192]

$[\text{deoxy}(\text{Hb}+\text{Mb})]_{\text{peak}}$ was also determined by Marzorati et al.[193] in a small population of patients with the adult form of Pompe disease (glycogen storage disease type II, due to the deficiency of the lysosomal enzyme acid maltase), before and after 12 months of enzyme replacement therapy. Following enzyme replacement therapy, the authors observed that $[\text{deoxy}(\text{Hb}+\text{Mb})]_{\text{peak}}$ slightly increased in some of the patients.

In patients with Friedreich's ataxia, the genetic defect decreases the expression of the protein frataxin, localized in mitochondria. The resulting impairment of mitochondrial function and oxidative metabolism has been identified at the skeletal muscle level by ^{31}P-MRS (see the discussion in Lynch[194]). These authors observed by NIRS a slower reoxygenation kinetics following exercise, and the degree of impairment of this variable correlated with the length of the triplet GAA repeat, a genetic measure associated with the age of onset of the disease.[194]

Quaresima and Ferrari[195] first investigated by NIRS quadriceps muscle oxygenation in patients with Duchenne muscular dystrophy (DMD). An insufficient increase in $\dot{Q}O_{2m}$ with respect to $\dot{V}O_{2m}$ occurs in these patients.[196] In other words, the functional defect is the opposite of that characterizing MM and McA patients (see above). The dystrophin deficiency results in a loss of sarcolemmal neuronal NO. NO plays a key role in the control of muscle blood flow during exercise, by blunting the vasoconstrictor response to α-adrenergic stimulation.[123,124] In DMD patients, the impaired NO synthesis would impair the intramuscular matching of $\dot{Q}O_{2m}$ versus $\dot{V}O_{2m}$, leading to an increased O_2 extraction, which has indeed been demonstrated by NIRS.[196] An enhanced O_2 extraction has been observed[197] also in patients with Becker muscular dystrophy, a milder form of dystrophy in which the intramuscular NO impairment is also present. In these patients, the enhanced O_2 extraction was associated with the functional impairment. An enhanced O_2 extraction, on the other hand, has not been described in patients with

limb-girdle muscular dystrophy, in whom neuronal NO synthase is not impaired.[196]

Caliandro et al.[198] could differentiate, by utilizing arterial occlusion testing in postocclusion hyperemia, patients with dermatomyositis and inclusion-body myositis from healthy controls; the differentiation was not possible for patients with polymyositis. A mitochondrial dysfunction, evaluated by the recovery kinetics of [PCr] (by ^{31}P-MRS) and $\dot{V}O_{2m}$ (by NIRS), was observed in patients with amyotrophic lateral sclerosis.[199]

5.5 *Other Diseases*

Other patients populations characterized by a skeletal muscle oxidative impairment, which was identified by NIRS by the analysis of the rate of decrease in $\dot{V}O_{2m}$ following voluntary or electrically stimulated contractions,[94,200] are those with cystic fibrosis[201] or spinal cord injuries.[202] In a case report, Ryan et al.[200] described a threefold increase of the rate of decrease in $\dot{V}O_{2m}$ (indicating a substantial improvement of skeletal muscle oxidative metabolism) in a patient with a spinal cord injury who underwent 12 weeks of endurance training, conducted by neuromuscular electrical stimulation.

Malenfant et al.[203] observed by NIRS, in patients with pulmonary arterial hypertension, markedly higher [deoxy(Hb+Mb)] during submaximal exercise at the same absolute work rate versus controls, indicating a markedly higher *vastus lateralis* fractional O_2 extraction. The data suggest an impaired $\dot{Q}O_{2m}$ in these patients, which would be compatible with the lower capillary density observed in the same muscle of the other leg. The data would also be compatible with the increases in endothelin-1 levels and with the decreases in NO and prostaglandins. Interestingly, hyperoxic breathing, while normalizing SO_{2a}, did not substantially affect the [deoxy(Hb+Mb)] response, confirming an impairment in $\dot{Q}O_{2m}$ deriving from the structural and functional impairments mentioned above.

In patients with fibromyalgia, Shang et al.[204] combined the utilization of a commercially available NIR oximeter with a custom-built NIR diffuse correlation spectroscopy (DCS) instrument for tissue $\dot{Q}_m$ measurements. Experiments were conducted during fatiguing knee extensors isometric contractions and a forearm arterial occlusion protocol. From the O_2 extraction and $\dot{Q}_m$ data, $\dot{V}O_{2m}$ was calculated (see also below). The patients showed, versus the controls, a lower fractional O_2 extraction and a slower reoxygenation kinetics.

Muscle NIRS measurement is an important tool in identifying high-risk patients in septic and hemorrhagic shock, by offering a continuous and noninvasive monitoring of tissue hypoperfusion.[205,206] According to these authors, during active shock resuscitation changes in skeletal muscle oxygenation levels are correlated with changes in O_2 delivery, base deficit, and blood lactate levels. A drop in muscle oxygenation levels predicts death and multiorgan failure, and may be helpful in making critical clinical decisions.

6 Instrumentation Advancements

The present section is aimed at briefly reporting the advancements made in the last few years in the field of NIRS instrumentations suitable for muscle studies and at mentioning the future directions of the development/utilization of these NIRS devices.

The main commercial NIRS oximeters for muscle studies, with the related key features and measurable parameters, are listed in Table 1. Since 2011 (when a homologous table was published in the review by Ferrari et al.[24]), different battery-operated portable/wireless muscle oximeters have been commercialized. The CW-NIRS portable/wearable systems, with wireless data transmission, are reported in Table 2. The details about each system can be found in the related company's websites.

Three different NIRS techniques are utilized in the field of muscle oximetry. Each of them is based on a specific type of illumination: (1) the most commonly used CW instruments, based on constant illumination of the tissue, simply measure the attenuation of light through the tissues; (2) FD instruments, illuminating the tissues with intensity-modulated light, measure both the attenuation and the phase delay of the emerging light; and (3) TD instruments, illuminating the tissue with short pulses of light (few picoseconds), detect the temporal broadening 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 muscle O_2 saturation (SO_{2m}, %). The SO_{2m} measurement assures an accurate quantitation of the oxygenation changes occurring at the skeletal muscle level. The 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, FD and TD techniques offer a better depth discrimination than that achieved by CW instruments and allow the absolute characterization of the tissue optical properties (absorption and reduced scattering coefficients), from which it is possible to retrieve absolute concentration values of [oxy(Hb+Mb)] and [deoxy(Hb+Mb)], and the derived parameter [total(Hb+Mb)]. The available CW-NIRS instruments do not determine reduced scattering coefficient and circumvent the problem by assuming that the coefficient is substantially constant during rest and exercise. This assumption has been criticized.[207] The issue has also been discussed in the review by Ferrari et al.[24]

Most of the NIRS instruments reported in Table 1 are represented by two to six channels and CW brain oximeters utilized also for physiological/pathophysiological muscle studies. These oximeters are the most commonly utilized NIRS instruments because they are of relatively low/moderate cost and transportable. Muscle NIRS instrumentations are characterized by a relatively high temporal resolution; the sampling rate is up to 100 Hz in some CW systems, 50 Hz in the only commercial FD system, and 0.2 Hz in the first TD oximeter with CE mark, commercialized in 2015 (Table 1). An original muscle oximeter has been very recently introduced by Reflectance Medical; the instrument collects muscle spectra and calculates SO_{2m} as well as pH_m.

The sector of wearable health technology is gaining increasing interest. The use of low cost wearable devices or wearable biosensors, which allow a constant monitoring of physiological signals, is essential for the advancement of both the diagnosis and the treatment of diseases, as well as for monitoring active life style. Very recently, a textile NIRS system has been utilized for monitoring arterial O_2 saturation (measured by pulse oximetry) and muscle oxygenation changes.[208] Wearable wireless fingertip pulse oximetry devices, for arterial O_2 saturation measurement, are already available for smart phones. In addition, a wireless and smartphone controllable NIRS system (DynaSense

Inc., Japan) has been very recently presented.[209] Table 2 includes also two wireless systems that are waterproof, then allowing muscle oxygenation measurements in swimmers.[210]

For a better understanding of skeletal muscle physiology, muscle oxygenation data should be ideally integrated with regional blood flow measurements. Direct and indirect NIRS methods to measure $\dot{Q}_m$[20,96] have been discussed above. A novel technique, known as DCS, has been recently developed for quantifying relative changes in microvascular regional blood flow. Basically, DCS uses coherent NIR light to penetrate deep tissues and measures speckle fluctuations of the diffuse light that are sensitive to the motions of red blood cells in tissues.[211,212] The well validated DCS provides a portable, noninvasive, and inexpensive alternative for measuring microvascular blood flow. A recent review article has described the progresses made in the field of DCS for assessing $\dot{Q}_m$ during dynamic exercise.[213] This technology allows also the estimation of skeletal muscle oxygenation levels. So far, two DCS-NIRS systems have already been commercially available: *αιμα*-FloMo (Hemophotonics S.L., Spain) and MetaOx (ISS, United States). The former measures only $\dot{Q}_m$, while the latest provides concomitant measurements of SO_{2m} and absolute [oxy(Hb+Mb)] and [deoxy(Hb+Mb)] by FD-NIRS. The method has been utilized on the head of newborns and on the skeletal muscles of adults. Giovannella et al.[214] have integrated the *αιμα*-FloMo with a TD-NIRS prototype (TRS20, Hamamatsu Photonics, Japan) to measure $\dot{Q}_m$ and absolute [total(Hb+Mb)] during quadriceps exercise. A different approach for measuring $\dot{Q}_m$ has been utilized by Ornim Inc. (United States), which has developed the brain/muscle oximeter "CerOx 3210F" using NIRS and weak acoustic beams to identify light emerging from deep tissue layers.

Many sophisticated multichannel CW-NIRS instrumentations, dedicated to brain cortex two-dimensional (2-D) imaging (topography), are commercially available and largely utilized in different areas in the field of neuroscience.[25,215] Unfortunately, the probes they are equipped with cannot be utilized for skeletal muscle 2-D imaging studies. In 2015, an eight-measurement point wireless system for 2-D brain/muscle measurements has been commercialized (Table 2). In the framework of a National Aeronautics and Space Administration (NASA) project, a 64-measurement point wireless NIRS system has been developed and integrated with simultaneous electrocardiography and actigraphy monitoring, in order to record 2-D muscle imaging data up to several days.[216] Although a three-dimensional (3-D) NIR imaging (tomography) of the human forearm, based on TD techniques, had been proposed[217] almost 15 years ago, no further consistent progresses in this field have been made. Recently, cross-sectional 2-D images of the foot [total(Hb+Mb)], using a multichannel 3-D CW system during thigh cuff occlusion, were reported.[218]

The state-of-the-art of TD-NIRS has been recently reviewed.[219] Many progresses have been done in the last 2 years that open a new way to compact, low-cost, and high-performance TD devices for diffuse muscle optical imaging and spectroscopy. The new frontiers in TD diffuse optics are covered by an article of this Special Section. [220] Briefly, consistent progresses are expected concerning noncontact scanning 2-D imaging systems for TD-NIRS based on a null source–detector separation.[221–223] These systems, already tested in the human forearm, utilize new fast detectors for extreme depth penetration and sensitivity.[224,225]

7 Conclusions

Researchers should always be critical and careful about the technologies and methods they utilize, but within reasonable limits. Many exercise physiologists, in our opinion, have been excessively critical about NIRS in the past. The method has important limitations but presents significant advantages compared to other methods. Skeletal muscle fractional O_2 extraction, the main variable that can be noninvasively evaluated by NIRS, can yield relevant information on key physiological and pathophysiological mechanisms, relevant in the evaluation of exercise performance and exercise tolerance in healthy subjects and in patients.

To put the method in a broader perspective it should be recognized, for example, that the BOLD phenomenon,[226] as obtained by functional magnetic resonance imaging, a functional neuroimaging technique which has profoundly revolutionized neuroscience with applications ranging from normal brain development, aging, brain disorders and diseases, is conceptually very similar to the fractional O_2 extraction variable determined by NIRS in skeletal muscle. Both variables, indeed, substantially reflect changes in tissue oxygenation resulting from the dynamic balance between tissue O_2 consumption and O_2 supply.

The recent diffusion of apparently easy-to-use portable NIRS instruments had led to the proliferation of studies, sometimes of dubious quality. An example is represented by studies, which claim to obtain absolute oxygenation indices when the instrumentation they employ does not allow this. The authors must be aware of the limitations of the method and should always have a clear and sound hypothesis to test. However, in the right hands (a good physiological background is mandatory), NIRS can offer insights into key physiological and pathophysiological adaptations to conditions of increased O_2 needs, often involving, in an integrated manner, different organs of the body. In terms of patients' evaluation, NIRS allows to determine the evolution of functional impairments of skeletal muscle oxidative metabolism with time, to identify correlations with clinical symptoms, to evaluate the effects of therapeutic or rehabilitative interventions, and to gain pathophysiological and diagnostic insights.

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Bruno Grassi, received his MD and PhD degrees and now is a professor of physiology in the Medical School of the University of Udine (Italy), where he is head of the Exercise Physiology Laboratory. His research interests are in the fields of exercise and environmental physiology, skeletal muscle bioenergetics, with special reference to oxidative metabolism, in health and disease. He is the author of 112 scientific publications on international peer-reviewed journals and of 25 chapters in books.

Valentina Quaresima received her PhD and now is an associate professor of biochemistry at L'Aquila University, Italy. She has pioneered clinical applications of NpIRS of the adult and infant muscle/brain and has given substantial contributions to wider NIRS scientific community. Since 1993, she has been involved in the study of localized measurements of muscular and cerebral oxygenation in humans by different NIRS techniques. She is the author of 100 scientific publications on international peer-reviewed journals.