Determination of Maximal Lactate Steady State in Healthy Adults: Can NIRS Help?

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ABSTRACT

BELLOTTI, C., E. CALABRIA, C. CAPELLI, and S. POGLIAGHI. Determination of Maximal Lactate Steady State in Healthy Adults: Can NIRS Help? *Med. Sci. Sports Exerc.*, Vol. 45, No. 6, pp. 1208–1216, 2013. **Purpose**: We tested the hypothesis that the maximal lactate steady state (MLSS) can be accurately determined in healthy subjects based on measures of deoxygenated hemoglobin (*deoxy*Hb), an index of oxygen extraction measured noninvasively by near-infrared spectroscopy (NIRS). **Methods**: Thirty-two healthy men (mean ± SD age = 48 ± 17 yr, range = 23–74 yr) performed an incremental cycling test to exhaustion and square wave tests for MLSS determination. Cardiorespiratory variables were measured bbb and *deoxy*Hb was monitored noninvasively on the right vastus lateralis with a quantitative NIRS device. The individual values of $\dot{V}O_2$ and HR corresponding to the MLSS were calculated and compared to the NIRS-derived MLSS ($NIRS_{MLSS}$) that was, in turn, determined by double linear function fitting of *deoxy*Hb during the incremental exercise. **Results**: $\dot{V}O_2$ and HR at MLSS were 2.25 ± 0.54 $L \cdot min^{-1}$ (76% ± 9% $\dot{V}O_{2max}$) and 133 ± 14 bpm (81% ± 7% HR_{max}), respectively. Muscle O_2 extraction increased as a function of exercise intensity up to a deflection point, $NIRS_{MLSS}$, at which $\dot{V}O_2$ and HR were 2.23 ± 0.59 $L \cdot min^{-1}$ (76% ± 9% $\dot{V}O_{2max}$) and 136 ± 17 bpm (82% ± 8% HR_{max}), respectively. For both $\dot{V}O_2$ and HR, the difference of $NIRS_{MLSS}$ from MLSS values was not significant and the measures were highly correlated ($r^2 = 0.81$ and $r^2 = 0.76$). The Bland–Altman analysis confirmed a nonsignificant bias for $\dot{V}O_2$ and HR (-0.015 $L \cdot min^{-1}$ and 3 bpm, respectively) and a small imprecision of 0.26 $L \cdot min^{-1}$ and 8 bpm. **Conclusions**: A plateau in muscle O_2 extraction was demonstrated in coincidence with MLSS during an incremental cycling exercise, confirming the hypothesis that this functional parameter can be accurately estimated with a quantitative NIRS device. The main advantages of $NIRS_{MLSS}$ over lactate-based techniques are the noninvasiveness and the time/cost efficiency.
Key Words: FUNCTIONAL EVALUATION, EXERCISE PRESCRIPTION, NONINVASIVE TECHNIQUES, ANAEROBIC METABOLISM

The maximal lactate steady state (MLSS) is defined as the highest exercise intensity that can be maintained over time without a continual blood lactate accumulation. It reflects the equilibrium between lactate production and utilization at the whole body level, i.e., the highest exercise intensity still compatible with constant lactate concentration in the peripheral blood (6). MLSS is a well-known index of endurance and training status (7). It can be used in both athletes and sedentary subjects to stratify individuals for their level of fitness, to classify the functional level of patients, to set training objectives, to determine exercise volume, and to monitor the results of specific interventions (2).

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The assessment of MLSS is somewhat invasive and "costly" because it requires an *ad hoc* time-consuming protocol. These characteristics make the test unsuitable for the application on a large scale, where simple, noninvasive, and relatively inexpensive as well as reliable methods are required. Several methods have been developed over the years to identify MLSS based on a simpler, more economical and less time-consuming approach. Yet, none of the currently available methods are free of faults. The direct methods, based on the determination of lactate concentration in blood (34), are also invasive and relatively time-consuming. The indirect methods, among which the gas exchange-based measures are the most commonly applied (3,38), are characterized by a lower accuracy compared to the direct ones, subjectivity in the determination, and they are prone to potentially large errors in subjects with irregular breathing pattern (38).

The near-infrared spectroscopy (NIRS) has been widely used in exercise physiology as a noninvasive technique for the functional evaluation of muscle oxidative metabolism (23). Muscle O_2 extraction, as measured by NIRS, changes linearly during an incremental cycling exercise, showing one or more modifications in slope (deflection points) between 40% and 80% of $\dot{V}O_{2max}$. A possible overlap of these deflection points with functional indexes related to anaerobic

metabolism has been suggested by previous studies conducted in different populations (healthy adults [5,17,24,32,37], chronic heart failure patients [24,36], and children [25]). All the studies come to the conclusion that a "threshold" intensity, correlated with the ventilatory threshold (5,25,37), the onset of blood lactate accumulation (17), or the respiratory compensation point (24), can be determined through noninvasive measures of muscle oxygen extraction during incremental exercise. Yet, the methodological differences among the studies and a suboptimal statistical approach (i.e., correlation analysis for the majority of studies) lead to incomparable and inconclusive results. Furthermore, the possible relationship between NIRS-derived "threshold" and the MLSS has never been evaluated.

In comparison to other technologies, the main strengths of NIRS-based measures are the noninvasive nature, the ability to evaluate small muscle masses, and the high sampling frequency that allows the characterization of the response to exercise in subjects with a limited exercise capacity. Furthermore, the re cently developed low-cost (29) and/or portable/wearable devices (19,23) allow the application of this technology on a large scale, in different types of effort and on the field.

By using a state-of-the-art quantitative NIRS device, *deoxy*Hb as an index of muscle O_2 extraction, in a relatively large, healthy, and heterogeneous male population, we tested the hypothesis that MLSS can be accurately determined in healthy subjects based on quantitative measures of *deoxy*Hb.

METHODS

Subjects

Thirty-two healthy sedentary males (anthropometric characteristics are presented on Table 1) were recruited by local advertisement in the metropolitan area of Verona. Inclusion criteria were between age 18 and 75 yr and male sex. Exclusion criteria were smoking, metabolic or cardiovascular conditions or the use of medications that may interfere with the physiological response to the tests (diabetes, high blood pressure, chronic heart failure, etc.), and double skinfold thickness on the lateral aspect of the thigh above 20 mm. In conformity to the principles of the Declaration of Helsinki, the study was approved by the Ethical Committee of the Department, and subjects were informed of the aims, the procedures, and the possible risks involved in the study and they gave a written consent. A medical evaluation preceded the inclusion in the study.

Protocol

On separate days, the subjects performed on the cycle ergometer: i) an incremental exercise to exhaustion and ii) three or four 30-min square wave exercises at increasing workload for MLSS detection, separated by a minimum interval of 48 h (4). All tests were performed at the same time of the day, after a standardized meal composed of 500 mL of water and 1–3 $g \cdot kg^{-1}$ of body weight of low-glycemic index CHO (the dose depending on the distance of the meal from the test) (i.e., 3 $g \cdot kg^{-1}$ for 3-h distance; 2 $g \cdot kg^{-1}$ for 2-h distance; 1 $g \cdot kg^{-1}$ for 1-h distance) (1) in comfortable and standardized ambient conditions (22°C–25°C and 55%–65% relative humidity).

Measures

All the exercise tests were performed on an electromechanically braked cycle ergometer (Excalibur Sport Device, Lode, the Netherlands), connected to and operated by a metabolic cart (Quark b^2; Cosmed, Rome, Italy) that also allowed continuous, breath-by-breath measures of pulmonary ventilation and gas exchanges at the mouth. Before each test, the gas analyzers and the turbine flow meter of the system were calibrated following the manufacturer's instructions and by using a gas mixture of known concentration (FO_2: 0.16; FCO_2: 0.05; N_2 as balance) and a 3.0-L calibrated syringe. HR was measured by means of a short-distance telemetry cardiotachometer interfaced with the metabolic cart. The cycle ergometer seat was adjusted to obtain a complete extension of the legs during pedaling, and the initial settings were maintained throughout the study. The subjects' preferred pedaling frequency was determined on the first test, and subjects were required to maintain it in all successive tests.

Incremental Exercise

It was preceded by a 3-min rest period. Thereafter, the workload was increased to 50 W for 3 min and then by 10–30 $W \cdot min^{-1}$. The increase in workload above the initial warm-up was chosen based on age and on an anticipation of the individual aerobic fitness with the aim to bring the subject to exhaustion within 8–12 min (2). The accepted criteria for maximal effort were RER > 1.1 and HR > 90% of the predicted maximum based on age (2). Should a test have not satisfied these criteria *a posteriori*, it was repeated.

Gas exchanges and HR were measured breath-by-breath throughout the exercise. During the incremental test, the muscle oxygen extraction was evaluated by means of a frequency-domain multidistance (FDMD) NIRS system (OxiplexTS™, ISS, Champaign, IL). After shaving, cleaning, and drying of the skin area, the NIRS lightweight plastic probe was longitudinally positioned on the belly of the vastus lateralis muscle, 15 cm above the patella and attached to the skin with a

TABLE 1. Mean ± SD and range values of age, weight, stature, body mass index (BMI), maximum oxygen consumption ($\dot{V}O_{2max}$) and the gas exchange threshold (GET) for the whole group of participants.

$n = 32$	Age (yr)	Weight (kg)	Stature (m)	BMI	$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	GET ($mL \cdot kg^{-1} \cdot min^{-1}$)
Mean ± SD	48 ± 17	76 ± 8	1.75 ± 0.09	25 ± 3	39.4 ± 11.4	21.5 ± 7.7
Range	23–74	62–98	1.56–1.90	20–31	21.8–59.8	10.1–39.5

biadhesive tape. The position of the probe on the thigh was then pen-marked to allow repositioning on the following appointments and to detect a possible sliding of the probe during the test. Finally, the probe was secured with elastic bandages around the thigh. The apparatus was calibrated on each test day, after a warm-up of at least 30 min, following the manufacturer's recommendations. The NIRS provides a continuous measurement (sampling frequency of 120 Hz) of absolute concentrations ($\mu mol \cdot L^{-1}$) of oxyhemoglobin (*oxy*Hb), *deoxy*Hb, total hemoglobin, and percent hemoglobin saturation.

Square Wave Exercises

On successive appointments, subjects performed three to four square wave tests, consisting of a 3-min rest, followed by a 30-min continuous exercise at constant workload. To reduce the number of exercise trials required for MLSS determination, the individual power output at the gas exchange threshold (GET; see Data analysis section) was used for the first square wave test. The intensity of the successive tests was defined based on the LA response to the first test, as described in Table 2.

Gas exchange variables and HR were measured breath-by-breath at rest and in the first 10 min of exercise, with the same equipment used for the incremental test. The blood lactate concentration ($[La]_b$, mmol) in arterialized capillary blood was assessed by means of an electroenzymatic method (Biosen C_line; EKF Diagnostic, Barleben, Germany) on 20-μL blood samples taken from an earlobe at rest and every 5 min throughout the exercise.

Data Analysis

Incremental exercise. The GET was determined based on the breath-by-breath data obtained from the incremental exercise (3). Furthermore, maximal parameters ($\dot{V}O_{2max}$, HR_{max}, and RER_{max}) were calculated as the average of the highest 10 s before the exhaustion.

Individual *deoxy*Hb data from the incremental exercise were averaged at 1 s and plotted as a function of time. The NIRS-derived MLSS ($NIRS_{MLSS}$) was identified by fitting the individual values of *deoxy*Hb corresponding to the incremental portion of the exercise (i.e., excluding the initial warm-up phase) as a function of time. *deoxy*Hb was preferred as an index of muscle oxygenation because it is less affected by changes in the volume of blood under the probe compared to other NIRS indexes (18,23). A double linear regression that minimized the squared sum of the residuals was then fitted by using a commercial software for data analysis (SigmaPlot 11.0; Systat Software, Inc., Chicago, IL) (Fig. 1):

$$f = \text{if } [x>\text{TD},\ g(x),\ h(x)]$$

$$g(x) = i_1 + (s_1 x)$$

$$i_2 = i_1 + (s_1 \text{TD})$$

$$h(x) = i_2 + [s_2(x - \text{TD})]$$

$$\text{fit } f \text{ to } y,$$

where f is the double linear function, x is time, and y is *deoxy*Hb; TD is the time coordinate corresponding to the interception of the two regression lines; i_1 and i_2 are the intercepts of the first and second linear functions, respectively; and s_1 and s_2 are the slopes.

On the basis of the cardiorespiratory data from the incremental exercise, the $\dot{V}O_2$ and HR at the time point corresponding to TD were calculated as the average of the last 10 s of the corresponding workload. These data identified the NIRS-derived MLSS ($NIRS_{MLSS}$).

Square wave exercises. MLSS was identified as the highest exercise intensity at which the difference between [LA] at 10 and 30 min was $<1\ mmol \cdot L^{-1}$ (4,6). $\dot{V}O_2$ and HR at this workload were calculated as a 30-s average at the 10th minute of exercise.

Statistics. Means ± SD were calculated for all parameters. A paired *t*-test was carried out to compare the average values of $\dot{V}O_2$ and HR at MLSS and $NIRS_{MLSS}$. Linear regressions and the Pearson product–moment correlation was used to verify the relationship between individual values of $\dot{V}O_2$ and HR at MLSS and $NIRS_{MLSS}$. The Bland–Altman analysis was applied to verify the agreement between measures (8).

Based on variances in $\dot{V}O_2$ measured in our laboratory (within-subject variation 2.5% ± 2.5%), using a power of 0.8 and an α level of 0.05, the sample size analysis for a paired *t*-test and correlation (Sigma Stat version 1; Jandel Scientific, S. Raphael, CA) indicated that the minimum number of subjects required to detect a significant difference (i.e., a 2.5% variation) was 10.

TABLE 2. Protocol of the square wave (SW) exercises used for detecting maximum lactate steady state (MLSS).

SW_1	Outcome	SW_2	Outcome	SW_3
Watt = GET	Stable [LA]	(SW_1) + 20%	Stable [LA]	(SW_1) + 30%
			Nonstable [LA]	(SW_1) + 10%
	Nonstable [LA]	(SW_1) − 20%	Stable [LA]	(SW_1) − 10%
			Nonstable [LA]	(SW_1) − 30%

For the first test (SW_1), a workload equal to the gas exchange threshold (GET) was imposed. Should a stable concentration of blood lactate ([LA]) result from the test (i.e., difference between [LA] at 10 and 30 min $< 1\ mmol \cdot L^{-1}$ [4,6]), then the workload of the successive square wave exercise (SW_2) would be equal to SW_1 + 20%. On the contrary, should an unstable [LA] result (i.e., difference between [LA] at 10 and 30 min $> 1\ mmol \cdot L^{-1}$ [4,6]), the workload for the successive exercise would be equal to SW_1 − 20%. In the case of a stable concentration of [LA] during SW_2, the workload of the successive square wave exercise (SW_3) was set to SW_1 + 30%. On the contrary, should an unstable [LA] result, the workload for the successive exercise would be equal to SW_1 + 10%.

RESULTS

The anthropometric characteristics (age, weight, stature, BMI, $\dot{V}O_{2max}$, and GET) of the subjects included in the study are reported in Table 1.

All the subjects completed the incremental cycling exercise, reaching on exhaustion, an HR_{max} equal to 95% ± 6% of age-predicted value (35) and an RER_{max} of 1.17 ± 0.09, both suggestive of a maximal effort, even if with a degree of imprecision (30). In the whole group, the $\dot{V}O_{2max}$ ranged from 22 to 60 $mL \cdot kg^{-1} \cdot min^{-1}$, the fitness level of the subgroups corresponding to the 80th, 50th, and 30th percentiles for young, middle age, and old adults, respectively (2). The GET (3), used to determine the workload for the first square wave exercise, was equal to 56% ± 10% $\dot{V}O_{2max}$ (Table 1).

No sliding of the NIRS probe was documented in all the tests. The *deoxy*Hb trend as a function of workload/time during an incremental or ramp exercise was detectable in all subjects: *deoxy*Hb increased very little in the warm-up phase of the test. Thereafter, in the incremental portion of the exercise, *deoxy*Hb increased linearly as a function of time and workload, up to a deflection point (i.e., a reduced slope or even a plateau) as a high-intensity exercise is approached (Fig. 1). The average value of *deoxy*Hb corresponding to the inflection point was 40 ± 16 $\mu mol \cdot L^{-1}$.

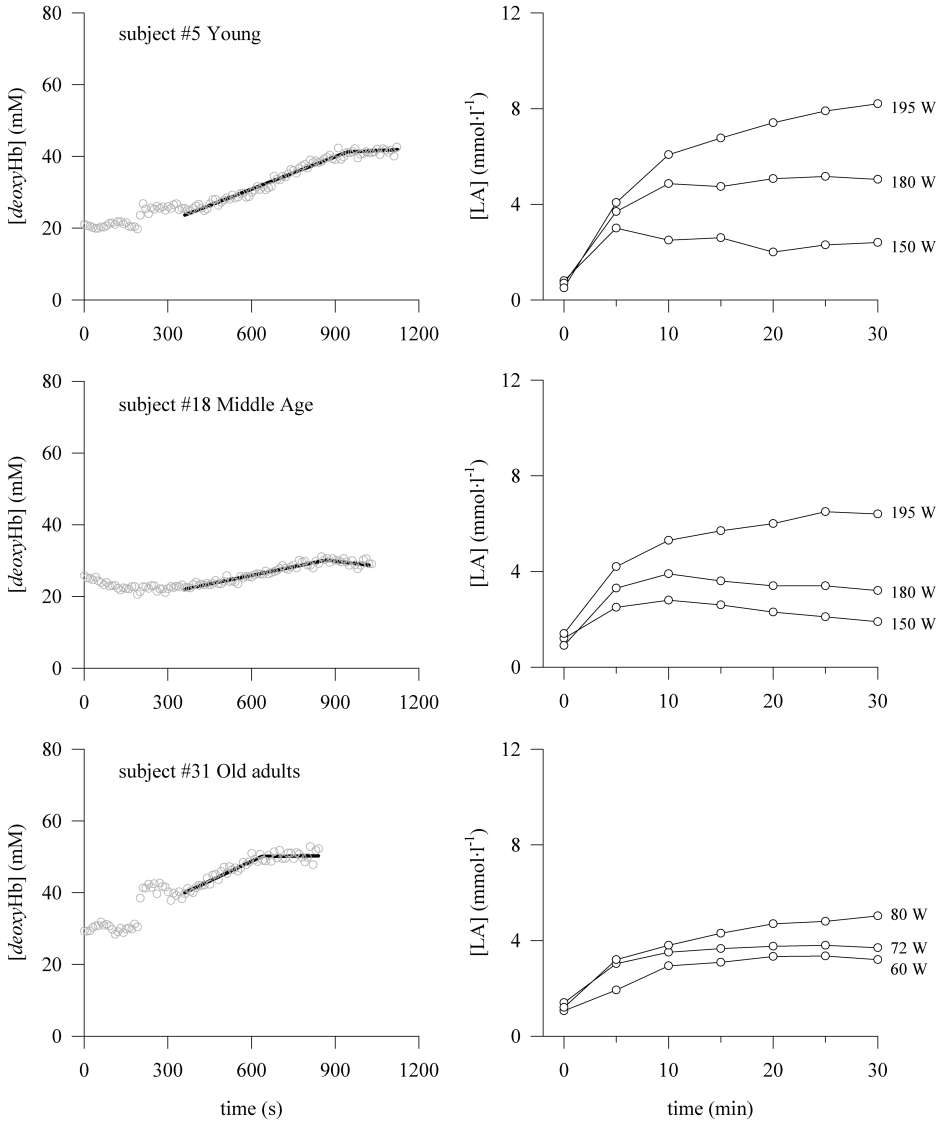

FIGURE 1—Left, The concentration of deoxygenated hemoglobin (*deoxy*Hb [$mmol \cdot L^{-1}$]) is plotted as a function of time (s) from the beginning of the incremental test up to exhaustion in three typical subjects (representative of the three age groups included in the study). The *black line* is the result of the double linear function fitting that was performed on the data of the incremental portion of the exercise (i.e., from the end of the warm-up phase up to exhaustion). The change in slope of the *deoxy*Hb signal as a function of time corresponds to the NIRS-derived maximal lactate steady state ($NIRS_{MLSS}$). Right, The concentration of lactate ([LA]) is plotted as a function of time (min) during three constant-load tests in the same three typical subjects. The highest workload still compatible with a constant [LA] (i.e., difference between [LA] at 10 and 30 min < 1 $mmol \cdot L^{-1}$ [4,6]) corresponds to the maximal lactate steady state.

TABLE 3. Mean ± SD at maximum lactate steady state (MLSS) and NIRS-derived MLSS ($NIRS_{MLSS}$) of oxygen consumption ($\dot{V}O_2$), percent maximal oxygen consumption ($\%\dot{V}O_{2max}$), HR, and percent maximal HR ($\%HR_{max}$).

	MLSS	$NIRS_{MLSS}$
$\dot{V}O_2$ ($L \cdot min^{-1}$)	2.25 ± 0.54	2.23 ± 0.59
$\%\dot{V}O_{2max}$	76 ± 9	76 ± 9
HR (bpm)	133 ± 14	136 ± 17
$\%HR_{max}$	81 ± 7	82 ± 8

All the subjects were able to complete the square wave exercises, and the MLSS was determined with no difficulty (Fig. 1). The mean value of lactate concentration at MLSS was 4.2 ± 1.1 $mmol \cdot L^{-1}$. The $\dot{V}O_2$ and HR (absolute and relative to $\dot{V}O_{2max}$ and to HR_{max}) at MLSS were not different from those at $NIRS_{MLSS}$ (Table 3). Both were significantly higher than the values measured at GET.

The values of $\dot{V}O_2$ and HR at MLSS were highly correlated with the same values found at $NIRS_{MLSS}$ (Fig. 2). Furthermore, the results of the Bland–Altman analysis (Fig. 3) showed that the mean difference (bias) between measures of MLSS and $NIRS_{MLSS}$ was -0.015 $L \cdot min^{-1}$ and 3 bpm for $\dot{V}O_2$ and HR, respectively, and not significantly different from zero. Finally, the SD (precision) was 0.26 $L \cdot min^{-1}$ and 8 bpm, while the 95% limits of agreement ranged from + 0.5 to -0.5 $L \cdot min^{-1}$ and from + 19 to -13 bpm for $\dot{V}O_2$ and HR, respectively.

DISCUSSION

We tested the possible correspondence between the traditional measure of MLSS and an innovative, noninvasive measuring technique, based on the detection of deoxygenated hemoglobin (*deoxy*Hb) deflection point (i.e., the NIRS-derived MLSS ($NIRS_{MLSS}$)), during an incremental cycling exercise.

The main finding of this study is that, in a relatively large and heterogeneous group of healthy males, both the $\dot{V}O_2$ and HR at the $NIRS_{MLSS}$ are not significantly different from, and they are highly correlated with, the values of the same variables at MLSS. Therefore, MLSS can be easily, rapidly, safely, and accurately determined during a standard incremental exercise to exhaustion on the cycle ergometer, by measuring *deoxy*Hb at the vastus lateralis, as a noninvasive index of muscle O_2 extraction.

Previous studies had suggested a possible utilization of the NIRS technology for the determination of landmarks of exercise intensity (5,17,24,32,36,37). Some studies demonstrated a correlation between a variety of NIRS indexes measured during incremental exercise and a variety of "thresholds." Yet, these studies differ in i) the site of measurement of NIRS signal (vastus lateralis [5,17,24,32,37] and respiratory muscles [25,36]), ii) the NIRS measuring devices (Runman NIM [5,25], NIRS HEO-100 [17], NIRO-200 Hamamatsu [37], Paratrend 7 Plus Diametrics Medical [32], OM-100A Shimadzu [24,36]), iii) the NIRS indexes of O_2 extraction (oxygenation index [5,25], deoxyhemoglobin [*deoxy*Hb] [37], oxyhemoglobin [*oxy*Hb] [17,24,36], interstitial fluid pH [32]), iv) the marker of "change" in the NIRS signal (decrease of *oxy*Hb below baseline value [5], first sharp decrease in oxygenation index [17], first and second inflection in *oxy*Hb [24], early increase in *deoxy*Hb slope [37], decreased interstitial fluid pH as a function of $\dot{V}O_2$ [32]), and v) the functional indexes (anaerobic threshold and respiratory compensation point by Wasserman method [24], ventilatory threshold by Beaver V-slope method [5,37], lactate threshold/onset of blood lactate accumulation by blood lactate determination [17] during a modified Bruce protocol [11]). In addition, the majority of the above studies have shown a correlation rather than a

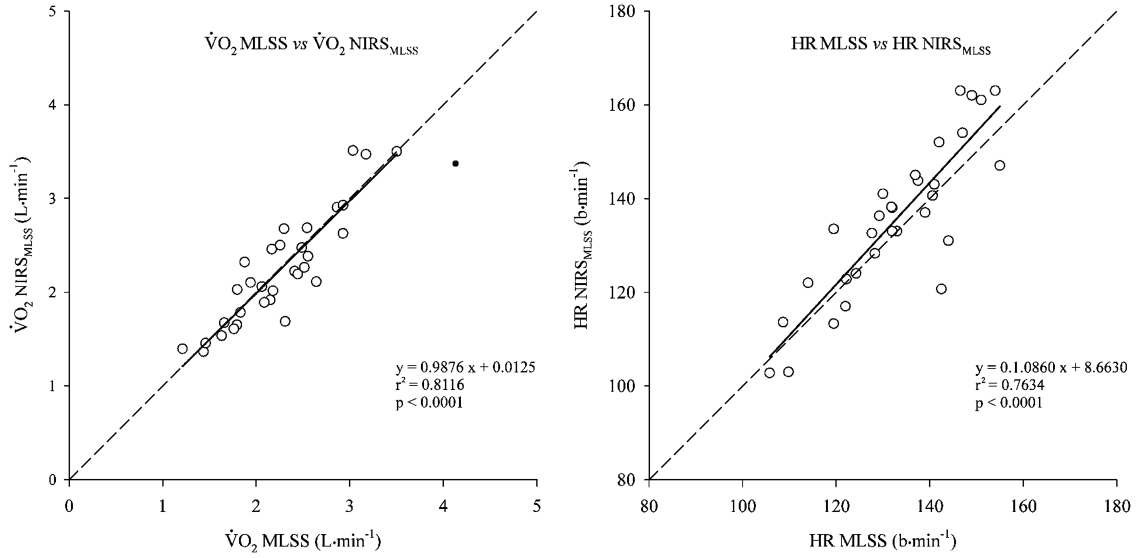

FIGURE 2—Correlation of $\dot{V}O_2$ (left graph) and HR (right graph) at maximum lactate steady state (MLSS) and NIRS-derived maximum lactate steady state ($NIRS_{MLSS}$): Individual values of $\dot{V}O_2$ (left) and HR (right) measured at $NIRS_{MLSS}$ during the incremental cycling exercise are plotted as a function of the same measure at MLSS in the whole group. The identity (*dashed*) and the regression (*solid*) line are displayed along with the regression equation parameters.

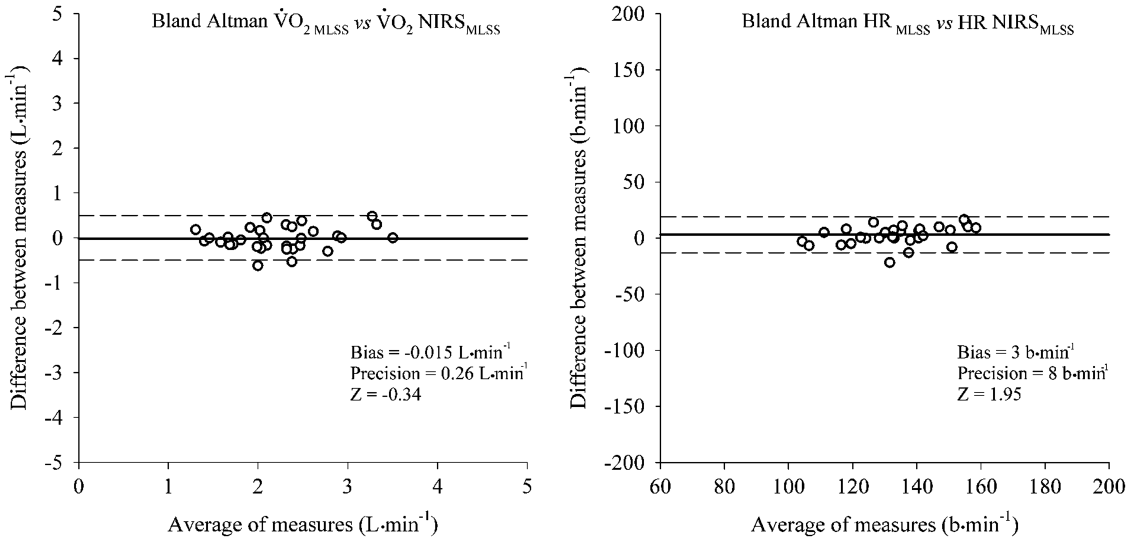

FIGURE 3—Bland–Altman plots of the $\dot{V}O_2$ (left graph) and HR data (right graph) related to $NIRS_{MLSS}$ and MLSS. In the graphs, individual differences between the MLSS and $NIRS_{MLSS}$ values are plotted as a function of the average of the two measures. The *solid line* corresponds to the average difference between measures (i.e., bias), while the *dashed lines* correspond to the upper and lower limits of agreement (precision).

coincidence between NIRS-derived indexes and the respective functional indexes. While all the studies conclude that an NIRS "threshold" intensity can be detected that is correlated with a variety of functional indexes, the use of different populations, different NIRS devices (some of which are presently obsolete), as well as different NIRS parameters and "threshold" indexes makes the direct comparison among the studies impracticable. Furthermore, a possible coincidence of NIRS "threshold" with MLSS had never been investigated.

In agreement with the literature, our data confirm the time course of *deoxy*Hb that has been observed during either ramp or incremental cycling exercises: after a first part characterized by little changes, *deoxy*Hb increases linearly up to approximately 70% $\dot{V}O_{2max}$; thereafter, either a reduced slope or an actual plateau is displayed (10,33). In our study, the $\dot{V}O_2$ measured at this deflection point ($NIRS_{MLSS}$) was not significantly different from the $\dot{V}O_2$ at MLSS ($P = 0.74$), the measures were highly correlated ($r^2 = 0.81$), and the bias between the measures was not significantly different from zero (-0.015 ± 0.26 L·min^{-1}). Furthermore, the HR at $NIRS_{MLSS}$ was not significantly different from the HR at MLSS ($P = 0.06$), the measures were highly correlated ($r^2 = 0.76$), and the bias between the measures was not significantly different from 0 (3 ± 8 bpm).

In summary, by using state-of-the-art quantitative NIRS technology, taking *deoxy*Hb as an index of muscle oxygenation and MLSS as the measure of the landmark intensity at which lactate production exceeds lactate removal, our study supports the hypothesis that MLSS can be determined accurately and precisely by this method in a relatively large sample of healthy adult males characterized by a broad range of fitness level.

Notwithstanding the coincidence between $NIRS_{MLSS}$ and MLSS, the possible physiological mechanism underpinning the relationship between the highest exercise intensity still compatible with constant lactate concentration in the peripheral blood and the deflection of *deoxy*Hb during an incremental trial remains to be elucidated.

Changes in the concentration of *deoxy*Hb, as measured by NIRS, are considered a proxy for microvascular O_2 extraction (14,18) and microvascular partial pressure of O_2 (PO_{2mv}) during exercise (20). Furthermore, while *deoxy*Hb signal cannot be used as a substitute for arteriovenous O_2 difference ($_{a\text{-}v}O_{2diff}$) (because the proportional contributions of arterial and venous blood to the overall signal are unknown), the two are considered to be related (22,33). For the above reasons, inferences into temporal changes in $_{a\text{-}v}O_{2diff}$ can be reasonably made, and according to the Fick principle, it can be assumed that the pattern of *deoxy*Hb during exercise can provide insight into the relationship between blood flow and oxygen uptake within the working muscles (9,33).

The time course of *deoxy*Hb during incremental exercises has been interpreted to reflect the balance between bulk and microvascular blood flow and muscle O_2 utilization that is influenced by the microvascular flow regulation and by muscle fibers recruitment pattern (9,12,16): the initial shallow slope of *deoxy*Hb has been attributed to a good matching of blood flow to O_2 extraction, thanks to a muscle pump effect and to the recruitment of predominantly Type I muscle fibers (characterized by a good matching of microvascular blood flow and muscle O_2 utilization). As work rate continues to increase, the steeper slope of *deoxy*Hb may be due to a slower adjustment in microvascular blood flow compared to muscle O_2 utilization and to a shift from predominantly slow-twitch muscle fibers to include more fast-twitch muscle fibers (9,16). A progressive metabolic acidosis could, in principle, contribute, through the Bohr effect, to an increased O_2 offloading from hemoglobin during this phase. Yet, a recent study by Boone et al. (10) suggested that

systemic metabolic acidosis *per se* (induced by previous high-intensity priming exercise performed with upper limbs) does not modify the time course of oxygen extraction during an incremental exercise performed with the lower limbs. On the contrary, oxygen extraction for a given workload is increased when prior high-intensity priming is performed with the lower limbs. The authors conclude that i) the Bohr effect is probably not the mechanism behind the sigmoid increase of *deoxy*Hb during a ramp exercise and ii) the increased oxygen extraction observed after lower limbs priming may be caused by the increased recruitment of fast-twitch fibers. In agreement with this view, Ferreira et al. (15) have shown that Type II fibers have a higher microvascular O_2 extraction as a function of exercise intensity compared to Type I fibers.

The reduced slope/plateau in *deoxy*Hb that characterizes the high-intensity portion of the incremental exercise would imply either a reduced O_2 utilization or an increased O_2 delivery. At high-intensity exercise, microvascular blood flow could be increased because of the accumulation of metabolites (H^+ ions, adenosine, and lactate) (21). Yet, the high level of force developed during each cycle may interfere with the muscle pump effect and dampen the increase in blood flow to the muscle at near-maximal exercise intensity (21). Therefore, we speculate that the reduced slope in *deoxy*Hb is unlikely to be due to an increased slope of blood flow over $\dot{V}O_2$ but may, on the contrary, suggest that O_2 extraction has an upper limit during dynamic exercise. In the severe-intensity domain, a plateau in the ability to use oxygen by slow, Type I fibers could be reached. Studies conducted in a rat model demonstrated that while the blood flow to the muscles continues to increase as a linear function of $\dot{V}O_2$, arteriovenous O_2 difference, on the contrary, is a hyperbolic function of $\dot{V}O_2$ (15). The reaching of a plateau could therefore be an indirect index of a shift toward an anaerobic ATP production to sustain muscle contraction. Alternatively, the reduced slope in *deoxy*Hb could be related to a larger and progressive recruitment of Type II fibers that have a low oxidative capacity but a high glycolytic capacity, compared to Type I fibers. As such, the increased contribution of glycolytic fibers to force production implies a progressively lower reliance on oxidative metabolism for ATP production. In agreement with this view, Ferreira et al. (15) have also shown that microvascular O_2 extraction in Type II fibers reaches a plateau at a lower exercise intensity compared to Type I fibers.

In summary, while the muscle continues to produce power at an increasing rate, a shift toward an increased reliance on anaerobic sources for ATP production (caused by a saturation of the ability of Type I fibers to use O_2 or by the larger contribution of Type II glycolytic fibers to force production or to a mix of both factors) could reduce the muscle's O_2 utilization rate. Under these conditions, although the total body $\dot{V}O_2$ may increase because of the contribution of other muscles (respiratory, trunk stabilizers, and other leg muscles), the ATP production within the working muscles may partially come from nonaerobic metabolic pathways (27). In agreement with the above speculation, Wilkerson et al. (39) documented a reduced gain in $\dot{V}O_2$ during square wave exercises in the severe-intensity domain.

The above explanations would be coherent with the finding of a coincidence between MLSS and $NIRS_{MLSS}$.

Fitting strategy. In literature, there are two main ways to fit *deoxy*Hb response as a function of the increase in exercise intensity during a ramp test: the double linear regression model (28,33) and the sigmoid model (9,10,16). Any attempt to characterize a wide range of possible response profiles using mathematical modeling is somewhat associated to limitations. A recent study suggested that the sigmoid model, which has been more traditionally used in this context, may not always characterize properly the phenomenological response in all subjects, specially so for the end-exercise portion of the experiment (33). The sigmoid approach is aimed at characterizing the overall response and it is based on the assumption that the lower and upper nonlinear segments of it are symmetrical. This assumption, at least in some cases, may jeopardize the ability to accurately characterize single portions of the response. For the above reasons, we favored the utilization of a double linear function that considered primarily the steep increase in *deoxy*Hb observed during the incremental exercise and the "plateau" phase that follows. A double linear model has been demonstrated superior in the description of the latter two components of the response of *deoxy*Hb during incremental exercise (33).

Critical power (CP) is thought to provide a noninvasive estimate of the maximal work rate that can be sustained in a full steady-state aerobic condition or maximal $\dot{V}O_2$ steady state (26). Furthermore, CP may represent the landmark intensity below which exercise can be sustained for a very long time without fatigue. A possible coincidence of MLSS with CP has been suggested, yet not univocally demonstrated, by several studies (13,31). In our study, we did not measure CP. Furthermore, we did not aim at describing $\dot{V}O_2$ kinetics during square wave exercises performed below, at, and above MLSS. Therefore, based on our data, we can neither confirm nor exclude that the $NIRS_{MLSS}$ could actually coincide with CP and with a maximal $\dot{V}O_2$ steady state. The possibility that MLSS, the reduced slope/plateau in O_2 extraction, and the maximal $\dot{V}O_2$ steady state occur together is very interesting and may allow some insights into the mechanisms that control/limit oxidative metabolism. Further research is needed to elucidate if CP actually coincides with MLSS and if it can be estimated based on the *deoxy*Hb deflection point during an incremental/ramp exercise.

Our data confirm the hypothesis that the MLSS can be accurately determined with this approach, using a quantitative NIRS. Along with the noninvasiveness, compared to lactate-based techniques, $NIRS_{MLSS}$ offers the advantage of being objective and independent from irregularities of breathing pattern that can heavily affect ventilatory-based techniques. In comparison to other technologies, the main

strengths of NIRS-based measures of MLSS are its noninvasive nature, its ability to evaluate even small muscle masses, and its high sampling frequency that allows the characterization of the response to exercise even in subjects with a limited exercise capacity and/or motivation. Furthermore, the recently developed low-cost (29) and/or portable/wearable devices (19,23) could allow the diffusion of this technology on a large scale, in different types of effort and on the field. The main limitation of a NIRS-based approach is related to its inability to evaluate the underlying muscle when a large fat layer (>30 mm) is present (23). Therefore, the methodological approach proposed in this study may be unsuitable for the evaluation of overweight/obese subjects and of a large portion of the female population.

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