Blood lactate accumulation and muscle deoxygenation during incremental exercise

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Grassi, Bruno, Valentina Quaresima, Claudioarconi, Marco Ferrari, and Paolo Cerretelli. Blood lactate accumulation and muscle deoxygenation during incremental exercise. *J. Appl. Physiol.* 87(1): 348–355, 1999.—Near-infrared spectroscopy (NIRS) could allow insights into controversial issues related to blood lactate concentration ($[La]_b$) increases at submaximal workloads ($\dot{w}$). We combined, on five well-trained subjects [mountain climbers; peak O_2 consumption ($\dot{V}O_{2peak}$), 51.0 ± 4.2 (SD) $ml \cdot kg^{-1} \cdot min^{-1}$] performing incremental exercise on a cycle ergometer (30 W added every 4 min up to voluntary exhaustion), measurements of pulmonary gas exchange and earlobe $[La]_b$ with determinations of concentration changes of oxygenated Hb ($\Delta[O_2Hb]$) and deoxygenated Hb ($\Delta[HHb]$) in the vastus lateralis muscle, by continuous-wave NIRS. A "point of inflection" of $[La]_b$ vs. $\dot{w}$ was arbitrarily identified at the lowest $[La]_b$ value which was >0.5 mM lower than that obtained at the following $\dot{w}$. Total Hb volume ($\Delta[O_2Hb + HHb]$) in the muscle region of interest increased as a function of $\dot{w}$ up to 60–65% of $\dot{V}O_{2peak}$, after which it remained unchanged. The oxygenation index ($\Delta[O_2Hb - HHb]$) showed an accelerated decrease from 60–65% of $\dot{V}O_{2peak}$. In the presence of a constant total Hb volume, the observed $\Delta[O_2Hb - HHb]$ decrease indicates muscle deoxygenation (i.e., mainly capillary-venular Hb desaturation). The onset of muscle deoxygenation was significantly correlated ($r^2 = 0.95$; $P < 0.01$) with the point of inflection of $[La]_b$ vs. $\dot{w}$, i.e., with the onset of blood lactate accumulation. Previous studies showed relatively constant femoral venous P_{O_2} levels at $\dot{w}$ higher than ~60% of maximal O_2 consumption. Thus muscle deoxygenation observed in the present study from 60–65% of $\dot{V}O_{2peak}$ could be attributed to capillary-venular Hb desaturation in the presence of relatively constant capillary-venular P_{O_2} levels, as a consequence of a rightward shift of the O_2Hb dissociation curve determined by the onset of lactic acidosis.

lactate threshold; near-infrared spectroscopy

THE QUESTION whether lactate accumulation in muscle and blood at submaximal workloads is attributable to an imbalance between O_2 supply and O_2 requirement in the working muscles, that is, to muscle hypoxia, is controversial (6, 14, 17). The issue is further complicated by the fact that lactate concentration in blood ($[La]_b$), as usually determined in the exercise physiology laboratory, cannot be considered a direct index of lactate production by muscles, because muscles, as well as other tissues and organs, are also consumers of lactate by oxidative metabolism for their energetic needs (6). In additon, lactate distribution throughout body compartments appears to be regulated by complex mechanisms (14, 16). Apart from its involvement in energy metabolism, other roles of lactate have been recently suggested. For example, according to Stringer et al. (35), lactic acidosis in muscle would facilitate O_2Hb dissociation and therefore increase O_2 extraction while preserving the O_2 pressure gradient from capillary to mitochondria.

Some further insights into these issues could be obtained by the utilization of near-infrared spectroscopy (NIRS), a noninvasive method that allows the monitoring of muscle oxygenation on the principle that the near-infrared light absorption characteristics of hemoglobin (Hb) and myoglobin (Mb) depend on their O_2 saturation [see, for instance, the recent reviews by Ferrari et al. (13) and by Mancini (22)]. Some of the limits of NIRS measurements are discussed below (see METHODS). In previous NIRS studies conducted during incremental exercise (3, 5, 24, 25, 37), $[La]_b$ values were not determined. Other authors evaluated the relationship between $[La]_b$ levels and muscle resaturation kinetics at the end of exercise (8) and described an association between muscle deoxygenation and $[La]_b$ levels during constant-load exercise (21) or between muscle deoxygenation and the so-called ventilatory threshold. To our knowledge, no formal comparison of NIRS-derived oxygenation indexes and $[La]_b$ has been performed during incremental exercise. The aim of this study was to fill this gap. More specifically, we intended to evaluate whether, during a standard incremental exercise conducted on a cycle ergometer, indexes of muscle oxygenation obtained by NIRS were associated with the onset of blood lactate accumulation or with some other parameters often employed to determine the so-called lactate threshold (LT).

METHODS

Subjects. The experiments were carried out in Milan, Italy (altitude ~150 m) on five men, who were well-trained mountain climbers [age, 32.8 ± 5.4 (SD) yr; height, 178 ± 11 cm; body weight, 73.3 ± 10.3 kg; blood Hb concentration ([Hb]), 14.7 ± 0.8 g/100 ml], ~3 wk before they participated in a Himalayan expedition to Mount Lhotse, Nepal (altitude 8,501 m). At the time of the tests, the subjects were not acclimatized to altitude, as can be deduced from their [Hb]. During the expedition, two of the subjects reached the summit, whereas the other three reached an altitude of ~8,000 m. All subjects did not utilize supplemental O_2 during the climb. The subjects gave their informed consent to participate in the study.

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All tests were performed under the supervision of a cardiologist.

Measurements. Measurements were carried out at rest and during an incremental exercise (starting from 60 W, 30 W were added every 4 min up to voluntary exhaustion) on an electrically braked cycle ergometer (Cardioline STS 3). No warm-up exercises were performed before the incremental test.

Pulmonary ventilation ($\dot{V}_E$, in BTPS), O_2 uptake ($\dot{V}_{O_2}$, in STPD), and CO_2 output ($\dot{V}_{CO_2}$, in STPD) were assessed on a breath-by-breath basis by a computerized system (Vmax 229, SensorMedics). $\dot{V}_E$ was calculated by integration of the flow tracings recorded at the mouth of the subject by a mass flow sensor. Volume calibration was performed before each experiment, by means of a 3-liter syringe, at three different flow rates. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were determined by continuously monitoring P_{O_2} and P_{CO_2} at the mouth of the subject throughout the respiratory cycle and from established mass balance equations. Calibration of the fast responding O_2 (paramagnetic) and CO_2 (nondispersive infrared) analyzers was performed before each experiment by utilizing gas mixtures of known composition. Heart rate (HR) was determined from the electrocardiogram, which was continuously monitored throughout the tests. Arterial blood O_2 saturation (Sa_{O_2}) was monitored continuously by pulse oximetry (Biox 3740 Pulse Oximeter, Ohmeda) at the earlobe. Average values of $\dot{V}_E$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, HR, and Sa_{O_2} were calculated during the last minute of rest and during the last 30–45 s of each workload. At rest and during the last 30–45 s of each workload, 20 μl of arterialized capillary blood were taken from an earlobe, and capillary $[La]_b$ was determined by an enzymatic method (ESAT 6661 Lactat, Eppendorf). No blood-gas measurements were performed.

Oxygenation of the vastus lateralis muscle was evaluated by continuous-wave NIRS. The theoretical basis, the principles of NIRS instrumentation, and the issue of quantification of NIRS data have been recently reviewed (9, 13, 22). The impossibility of obtaining quantitatively accurate values of tissue oxygenation, even by utilizing the most sophisticated "quantitative" algorithms of the intensity-modulated instruments (7), represents an intrinsic limit of optical methods that assume homogeneity of examined tissue volumes. Nonetheless, thanks to their portability, two-wavelength continuous-wave NIRS instruments have been widely utilized to obtain relative measurements of muscle oxygenation during dynamic exercise in humans (3–5, 8, 21–24, 31, 37). The method has been tested in vitro (8) and on tissuelike phantoms (19) as well as in vivo on different experimental animal and human models, correlating NIRS-derived oxygenation measurements with oxygenation of venous blood samples (19, 23, 28). More specifically, the differential absorption between 760 and 800 nm of light (taken as an oxygenation index) measured from the surface of exercising canine gracilis muscle was highly correlated with venous Hb O_2 saturation (37). The same conclusion applied to human forearm exercise (23). More recently, a portable two-wavelength continuous-wave NIRS instrument (HEO-100, OMRON), which utilizes an algorithm based on diffusion theory (33), has been developed, and it offers the advantage of providing separate measurements of changes in deoxygenated Hb (Δ[HHb]) and oxygenated Hb concentration (Δ[O_2Hb]), although expressed in arbitrary units (homologous across subjects), thereby providing a semi-quantitative evaluation of muscle oxygenation. This instrument was utilized for the present study. The probe unit, molded in elastic black silicone rubber, has a silicon photodiode as a photodetector in the center and two light-emitting diodes (peak wavelengths 760 and 840 nm) on either side. The probe was firmly attached to the skin overlying the lower one-third of muscle (~10–12 cm above the knee joint), parallel to the major axis of the thigh, by a belt secured by Velcro straps and biadhesive tape. The skin was carefully shaven previously. Pen marks were made over the skin to indicate the margins of the belt to check for any downward sliding of the probe during cycling. No sliding was observed in any subject. Black cloths were put around the probe and the skin to prevent contamination from ambient light. The probe was connected to a personal computer for data acquisition, analog-to-digital conversion, and subsequent analysis. The sampling frequency was set at 2 Hz. The distance between each light source and the photodiode was 3 cm. Thus the penetration depth can be estimated to be ~1.5 cm, as extensively discussed previously (10, 15). The influence of subcutaneous adipose tissue on near-infrared light propagation in leg muscle and on the sensitivity of NIRS instruments has been recently investigated by ultrasound (15). Those authors demonstrated that the near infrared light penetrates shallow regions of muscle (~2–4 cm^3 under the skin and subcutaneous fat) even when the adipose tissue thickness is 1.5 cm. Transmitted NIR light penetrates skin, subcutaneous fat, and underlying muscle and is either absorbed or scattered within the tissues. Part of the scattered light is detected by the photodetector. The absorption characteristics of light at 760 and 840 nm depend on relative oxygenation of Hb and Mb. Mb indeed has similar absorption spectra to Hb. In human skeletal muscle, however, the ratio of [Hb] to [Mb] is >5 (20, 22) so that the signals can be considered as deriving mainly from Hb. This was also suggested by studies conducted by simultaneous use of proton magnetic resonance spectroscopy (which allows in vivo detection of deoxygenated Mb) and NIRS in exercising humans (24). NIRS-obtained oxygenation values represent volume-averaged values in the segment of tissue under consideration.

Δ[O_2Hb] and Δ[HHb], with respect to an initial value arbitrarily set equal to zero, were calculated and expressed in arbitrary units (33). The sum between the two variables (Δ[O_2Hb + HHb]) is related to changes in the total Hb volume in the muscle region of interest, whereas the difference between the two variables (Δ[O_2Hb − HHb]) was taken as an oxygenation index (see also RESULTS). Average Δ[HHb], Δ[O_2Hb], Δ[O_2Hb + HHb], and Δ[O_2Hb − HHb] values were calculated at rest and during the last 30–45 s of each workload to obtain steady-state values aligned in time with blood sampling for $[La]_b$ determination.

Skinfold thickness at the site of application of the NIR probe was determined at the end of the exercise protocol by a caliper (Holtain). The obtained values were 5.3 ± 1.3 mm (range, 4.5–7.5 mm).

Statistical analysis. Data were expressed as means ± SD. Regression and correlation analyses were performed by the least squares method by utilizing a commercially available software package (InStat, GraphPad Software). The level of significance was set at $P < 0.05$.

RESULTS

Peak values obtained during the exhausting workload (288 ± 40 W) for ventilatory, metabolic, and cardiovascular parameters were $\dot{V}_E$, 119.6 ± 14.5 l/min; $\dot{V}_{O_2}$, 3.71 ± 0.40 l/min (51.0 ± 4.2 $ml \cdot kg^{-1} \cdot min^{-1}$); $\dot{V}_{CO_2}$, 3.73 ± 0.54 l/min; Sa_{O_2}, 94 ± 3%; HR, 182 ± 11 beats/min; and $[La]_b$, 8.5 ± 1.6 mM.

Individual Sa_{O_2} values are shown in Fig. 1 as a function of workload. Three of the subjects showed some arterial desaturation at the exhausting workload.

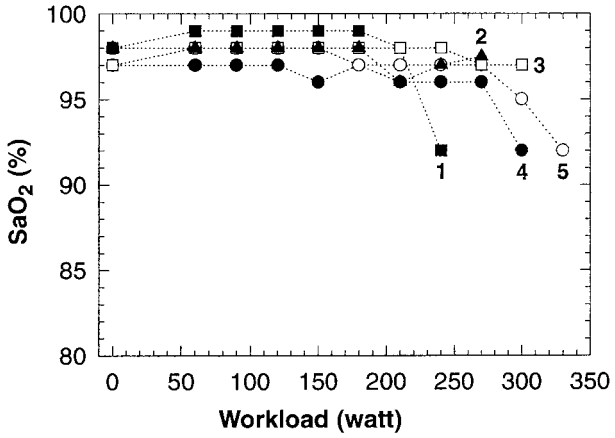

Fig. 1. Individual values, for *subjects 1–5*, of arterial blood O_2 saturation (Sa_{O_2}), as determined by earlobe pulse oximetry, as a function of workload.

Individual values obtained by NIRS are shown in the *top* and *middle* panels of Figs. 2-6 as a function of workload. For each subject, *top* panels show Δ[HHb] and Δ[O_2Hb] values, whereas *middle* panels show Δ[O_2Hb + HHb] and Δ[O_2Hb − HHb] values. In all subjects Δ[O_2Hb + HHb] underwent a slight linear increase as function of workload, up to 60–65% of the exhausting workload, after which the variable remained substantially unchanged. The Δ[O_2Hb + HHb] increase up to 60–65% of the exhausting workload was mainly attributable to increased Δ[HHb], because Δ[O_2Hb] was substantially constant in this workload range. In all subjects, Δ[O_2Hb − HHb] decreased with increasing workload. According to visual inspection, the pattern of decrease appeared to be characterized by two linear functions with increasing slopes. The workload at which the change in slope occurred was determined by iteratively fitting different combinations of two linear regressions to contiguous experimental points obtained during exercise and by evaluating which combination yielded the lowest sum of squared residuals. Resting values were not utilized for this analysis because, as pointed out by Maehara et al. (21), in resting subjects, the NIRS signal could be significantly contaminated by tissues other than skeletal muscle, such as the skin. One subject (*subject 1*) showed a leveling off in Δ[O_2Hb − HHb] decrease at the highest workload. This value was not utilized for the regression analysis. The combinations of linear regressions with the lowest sum of squared residuals are shown in Figs. 2–6. A point of inflection of muscle deoxygenation was identified at the workload at which the change in slope of Δ[O_2Hb − HHb] occurred. These points of inflection are indicated by the arrows in the *middle* panels of Figs. 2–6. The points of inflection corresponded also to the workload at which Δ[O_2Hb] started to decrease. It should be noted that Δ[O_2Hb − HHb] can be considered a reliable oxygenation index only if Δ[O_2Hb + HHb] is constant. In the present study, muscle deoxygenation [as indicated by the accelerated Δ[O_2Hb − HHb] decrease that begins at 60–65% of peak O_2 consumption ($\dot{V}O_{2peak}$)] was described in the presence of a constant Δ[O_2Hb + HHb] (Figs. 2–6, *middle* panels), thereby indicating a true deoxygenation. With a constant total Hb volume, the increase in Δ[HHb] and the decrease in Δ[O_2Hb] (Figs. 2–6, *top* panels) suggest, indeed, an imbalance between O_2 delivery and O_2 demand in the region of tissue under consideration. For workloads <60–65% of $\dot{V}O_{2peak}$, Δ[O_2Hb + HHb] showed a slight linear increase (see Figs. 2–6, *middle* panels). In this workload range, the observed slight decrease in Δ[O_2Hb − HHb] cannot be considered an indication of muscle deoxygenation, because the Δ[O_2Hb + HHb] increase was attributable to a Δ[HHb] increase, whereas Δ[O_2Hb] was unchanged (Figs. 2–6, *top* panels), thereby

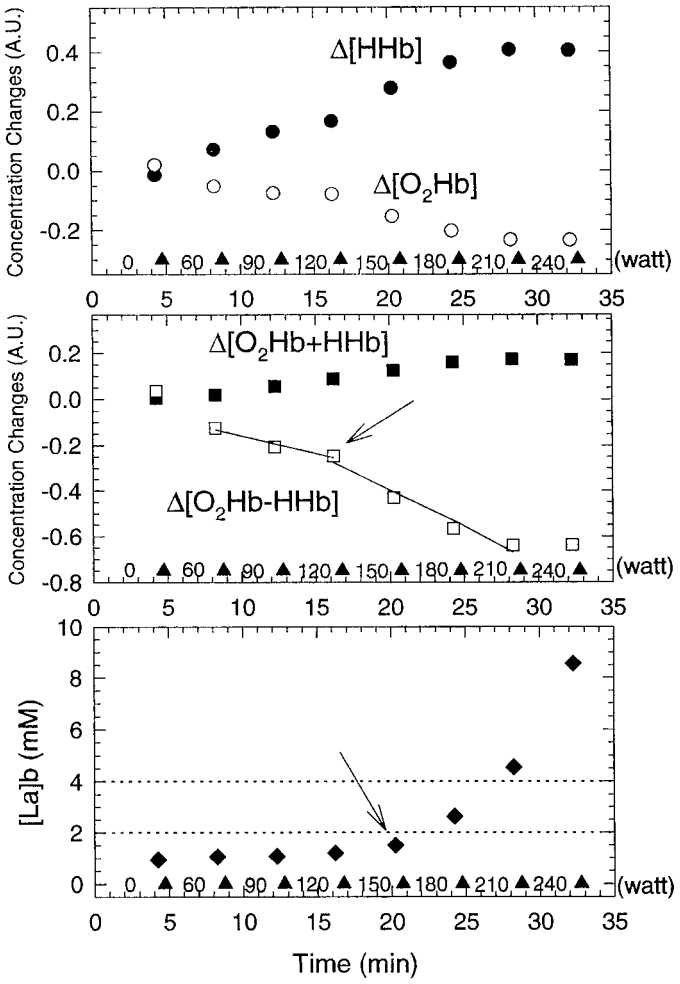

Fig. 2. Near-infrared spectroscopy (NIRS) indexes of vastus lateralis muscle oxygenation (*top* and *middle*) and capillary blood lactate concentration ($[La]_b$; *bottom*) are shown as a function of exercise time and workload for *subject 1*. NIRS indexes are expressed in arbitrary units (AU) as concentration changes with respect to an initial value set to equal zero. *Top*: concentration changes of oxygenated Hb (Δ[O_2Hb]) and deoxygenated HB (Δ[HHb]). *Middle*: sum (Δ[O_2Hb + HHb]), or total Hb volume in the region of interest, and difference (Δ[O_2Hb] − HHb), or oxygenation index, between the 2 variables of *top* panel. A change in slope in Δ[O_2Hb − HHb] decrease during exercise was identified by calculating combinations of linear regressions (lines shown in figure) that yielded the lowest sum of squared residuals. Arrow, point of inflection of Δ[O_2Hb − HHb] decrease. *Bottom*: point of inflection (arrow) of $[La]_b$ vs. workload was arbitrarily identified at lowest $[La]_b$ value that was >0.5 mM lower than the following one. See text for further details.

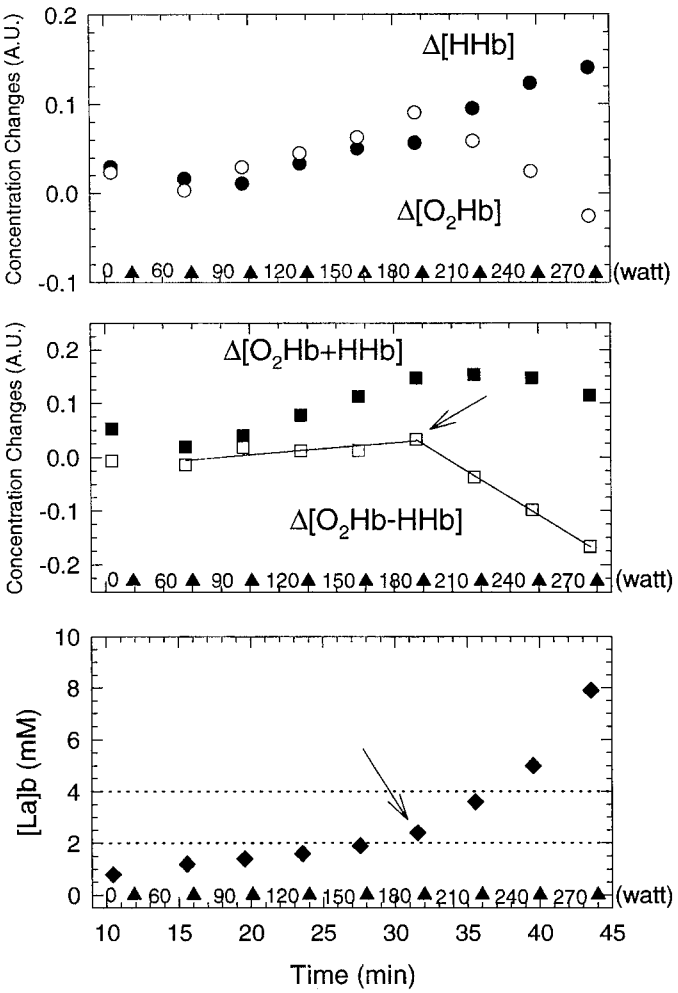

Fig. 3. Data for *subject 2*, as described in Fig. 2.

suggesting capillary-venular vasodilation. Unfortunately, no mathematical methods that account for Hb volume changes in the interpretation of deoxygenation indexes are available. From the above discussion, however, it would seem that, in terms of muscle oxygenation, the change in slope at 60–65% of $\dot{V}O_{2peak}$ would be even more pronounced than that observed for $\Delta[O_2Hb - HHb]$. The fact that onset of muscle deoxygenation occurred at about the same percentage of $\dot{V}O_{2peak}$ (60–65%) at which $\Delta[O_2Hb + HHb]$ leveled off, after a steady increase at lower workloads, suggests that muscle deoxygenation occurred after the exercise-related vasodilatatory capacity within the muscle reached its maximum.

Individual values of $[La]_b$ vs. workload are shown in the *bottom* panels of Figs. 2–6. In these panels, horizontal lines indicate the fixed values of $[La]_b$ (2 and 4 mM) that are conventionally used to determine the so-called lactate threshold, or LT (1). $[La]_b$ showed the classic curvilinear pattern of increase as a function of workload. In recent years, several mathematical models were applied to describe this pattern of increase (27) and to confirm or confute the existence of a threshold. In the present study, we were simply interested in identification of the workload at which lactate started to accumulate significantly in blood. For descriptive purposes, without making any inference on the mechanisms involved, we chose to identify a point of inflection of $[La]_b$ vs. workload as the lowest $[La]_b$ value that was >0.5 mM lower than the following one, i.e., according to an empirical method recently suggested by Zoladz et al. (38). These points of inflection of $[La]_b$ vs. workload are indicated by the arrows in Figs. 2–6, *bottom* panels. In four of five subjects, the point of inflection of $[La]_b$ vs. workload was the same as the point of inflection of muscle deoxygenation. In one subject (*subject 1*), the point of inflection of muscle deoxygenation was 30 W lower than the point of inflection of $[La]_b$ vs. workload. The highest workload corresponding to $[La]_b$ values lower than or equal to 2 or 4 mM was also determined. These workload values were termed LT ≤ 2 mM and LT ≤ 4 mM. The points of inflection of $[La]_b$ vs. workload, LT ≤ 2 mM and LT ≤ 4 mM were plotted for each subject as a function of the point of inflection of muscle deoxygenation (Fig. 7). The identity line between the variables is also shown in the figure. A significant correlation (r^2 = 0.95; P = 0.0045) was observed only between the point of inflection of $[La]_b$ vs. workload and the point of inflection of muscle deoxygenation. Figure 7 shows that LT ≤ 4 mM clearly overesti-

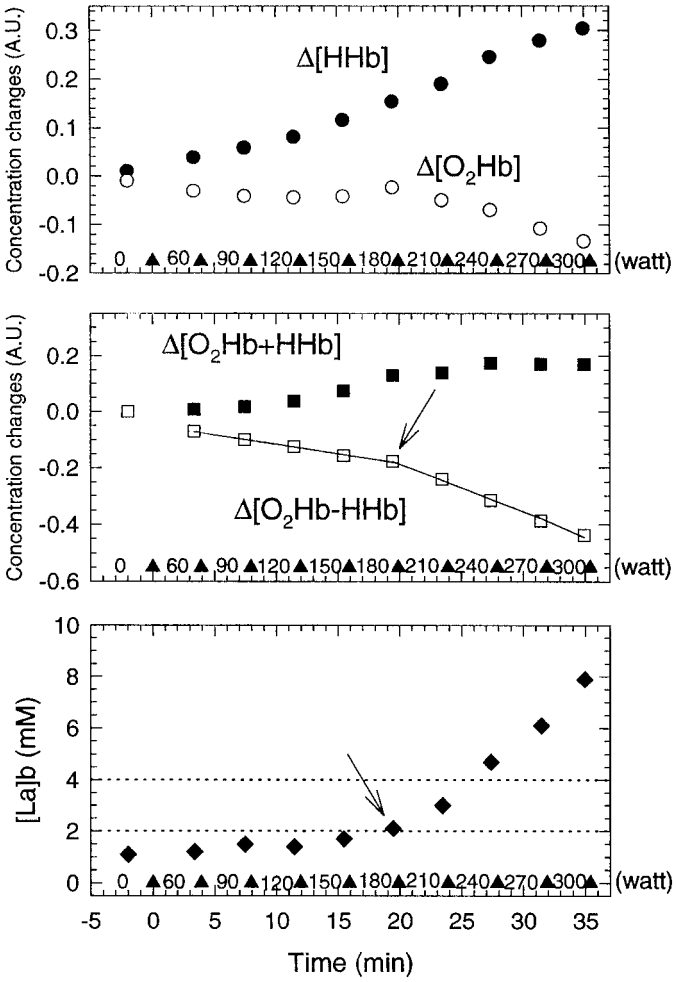

Fig. 4. Data for *subject 3*, as described in Fig. 2.

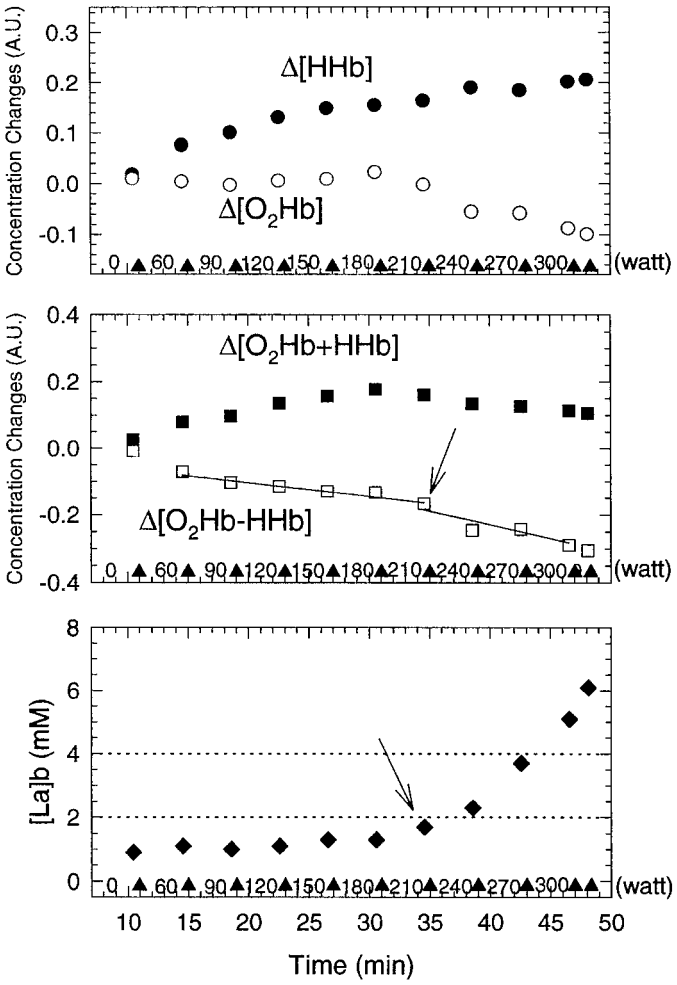

Fig. 5. Data for *subject 4*, as described in Fig. 2.

mated the point of inflection of muscle deoxygenation. The point of inflection of muscle deoxygenation occurred at 62 ± 7% of $\dot{V}O_{2peak}$, the point of inflection of $[La]_b$ vs. workload was at 64 ± 4% of $\dot{V}O_{2peak}$, LT ≤ 2 mM was at 62 ± 5% of $\dot{V}O_{2peak}$, and LT ≤ 4 mM was at 80 ± 7% of $\dot{V}O_{2peak}$.

DISCUSSION

In recent years, NIRS has been used rather extensively to monitor oxygenation changes in working muscles (3–5, 8, 21, 23, 24, 31, 37). This noninvasive method is based on the principle that the light absorption characteristics of Hb and Mb in the NIR region change depending on their O_2 saturation (7, 9, 13, 22). Some intrinsic limitations of this method were discussed above (see METHODS). NIR light-absorption changes in muscle reflect changes in oxygenation at the level of small blood vessels (small arterioles and venules), capillaries, and intracellular sites of O_2 transport and uptake (23). As mentioned above, NIRS cannot differentiate between absorption changes due to Hb and Mb, because the absorption spectra of the two molecules are the same. However, the ratio of [Hb] to [Mb] in skeletal muscle is >5 (20, 22) so that most of the absorption changes can be considered to be derived mainly from Hb, as supported also by studies conducted by simultaneous use of proton magnetic resonance spectroscopy (which allows in vivo detection of deoxygenated Mb) and NIRS in exercising humans (24). During incremental exercise, some endurance athletes show variable degrees of arterial desaturation at maximal workload (11). This was the case also for the present study, as shown in Fig. 1. On the basis of such premises, it appears reasonable to assume that muscle desaturation observed in the present study, beginning at 60–65% of $\dot{V}O_{2peak}$, indicated mainly a Hb desaturation occurring at the capillary and venular level.

The main finding of the present study was that, during an incremental exercise on a cycle ergometer, the onset of blood lactate accumulation {arbitrarily defined as the lowest $[La]_b$ value that was >0.5 mM lower than the following one (see RESULTS)} was significantly correlated with the onset of muscle (or, more precisely, capillary-venular) deoxygenation, as assessed by NIRS. Muscle deoxygenation could, in theory, be attributed to an accelerated fall of capillary-venular P_{O_2}, occurring in association with the appearance of lactate in blood. However, several papers (30, 35) reported that, during incremental exercise, the measured femoral venous P_{O_2} (considered an index of

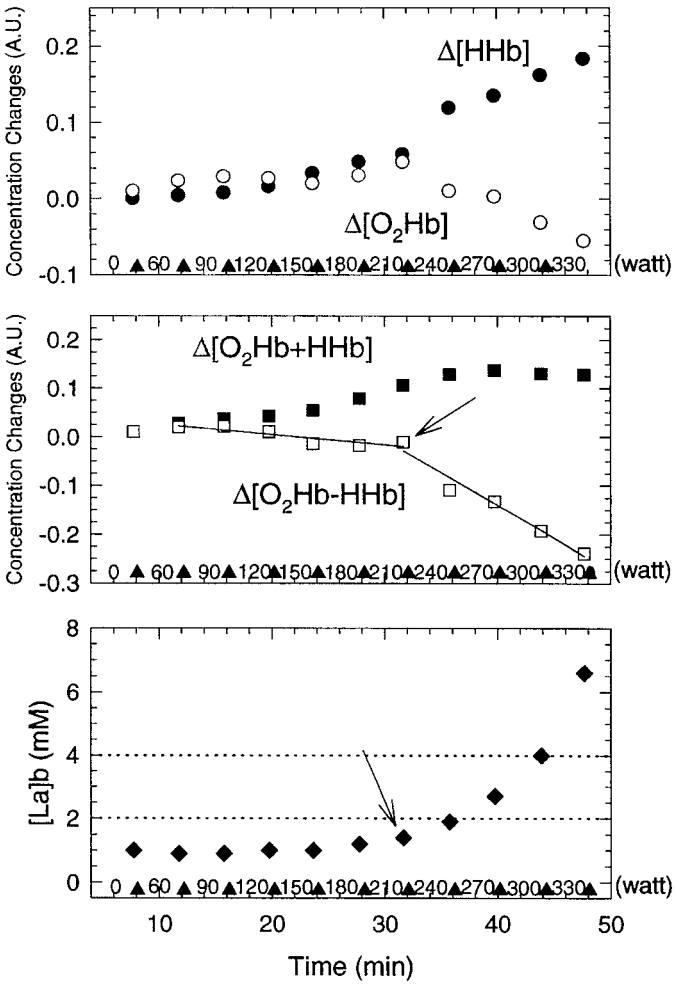

Fig. 6. Data for *subject 5*, as described in Fig. 2.

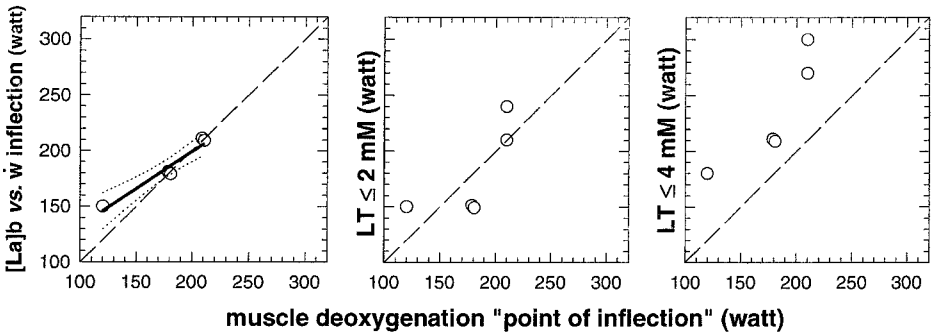

Fig. 7. Points of inflection of $[La]_b$ vs. workload ($\dot{w}$) and the highest workload associated with $[La]_b \leq 2$ mM lactate threshold (LT) or ≤4 mM LT plotted as a function of points of inflection of muscle deoxygenation. Each symbol represents value for 1 subject. Dashed lines, identity lines. *Left*: a significant correlation ($r^2 = 0.95$; $P = 0.0045$) was observed between the points of $[La]_b$ vs. workload and the points of inflection of muscle deoxygenation [regression line (solid line) and ±95% confidence intervals (dotted lines) are shown]. *Middle* and *right*: no significant correlations were observed between the muscle deoxygenation and LT ≤2 mM or LT ≤4mM. See text for further details.

end-capillary P_{O_2}) or the calculated mean capillary P_{O_2} show, after an initial abrupt decrease that occurs at low workloads, a tendency to level off (at a femoral venous P_{O_2} of ~17–20 Torr) at workloads >60% of $\dot{V}_{O_{2max}}$. Stringer et al. (35), in particular, showed that the leveling off of femoral venous P_{O_2} occurred at workload just above the so-called ventilatory threshold (2). Thus muscle deoxygenation observed in the present study at workloads >60–65% of $\dot{V}_{O_{2peak}}$ is likely attributable to an accelerated capillary-venular Hb desaturation in the presence of relatively constant capillary-venular P_{O_2} levels. This would imply a rightward shift of the O_2Hb dissociation curve as a consequence of lactic acidosis. This is in agreement with data obtained by Systrom et al. (36), who showed an abrupt decrease of exercising calf muscle pH (determined by ^{31}P-nuclear magnetic resonance spectroscopy) at ~65% of $\dot{V}_{O_{2max}}$. Such a pH threshold occurred at a workload that was not significantly different from that associated with the LT (determined by these authors by a log-log plot of venous [La] vs. $\dot{V}_{O_2}$). A rightward shift of the O_2Hb dissociation curve could also derive from increased muscle temperature. According to Saltin and Hermansen (32), however, muscle temperature is linearly related to workload, so a temperature increase should not be responsible for muscle deoxygenation that occurs abruptly at 60–65% of $\dot{V}_{O_{2peak}}$. As pointed out by Stringer et al. (35), the rightward-shifted O_2Hb dissociation curve would allow an increased O_2 extraction, but at the same time it would prevent large falls in capillary P_{O_2}, the driving force for peripheral O_2 diffusion. A well-preserved peripheral O_2 diffusion would be compatible with data recently obtained by Richardson et al. (29), according to which human muscle cytoplasmic P_{O_2} (calculated on the basis of Mb saturation, measured by proton magnetic resonance spectroscopy) is kept constant at an average value of ~3 Torr during exercises from 50 to 100% of $\dot{V}_{O_{2max}}$. These authors did not observe a correlation between cytoplasmatic P_{O_2} and lactate efflux from the exercising muscle. On the other hand, Duhaylongsod et al. (12) observed a negative linear relationship between the relative concentration of oxidized cytochrome a,a_3 (as determined by NIRS) and lactate efflux from canine gracilis in vivo. In the present study, our data showed only a significant correlation between the onset of muscle (capillary/venular) deoxygenation and the onset of blood lactate accumulation and do not allow inferences on any cause-effect relationship between the two variables. A clearer picture could be derived by future studies aimed at evaluation of relationships between the two variables during incremental exercises in acute hypoxia and hyperoxia. The present data do not allow either inferences on the issue of adequate vs. inadequate mitochondrial O_2 levels in the regulation of glycolysis.

Previous NIRS studies conducted during incremental exercise (3, 5, 24, 25, 37) did not assess blood lactate concentration. The study by Chance et al. (8) evaluated the relationship between blood lactate levels and the resaturation kinetics of Hb at the end of exercise. Belardinelli et al. (3) and Bhambhani et al. (5) utilized NIRS during incremental exercise and compared the observed pattern of deoxygenation with the so-called ventilatory threshold (2). Although the patterns of deoxygenation were different in the two studies, both groups claimed that they were closely related to the ventilatory threshold. In both studies, $[La]_b$ values were not assessed. The pattern of deoxygenation observed in the present study appears similar to that described by Belardinelli et al. (3) in a group of trained and untrained subjects. Also these authors described an accelerated muscle deoxygenation that occurred at ~50% of $\dot{V}_{O_{2max}}$, in close correlation with the ventilatory threshold. Taken together, the results of the present study and those of Belardinelli et al. suggest a close relationship between NIRS-derived indexes of muscle oxygenation and the ventilatory threshold or LT, when the latter is defined as the lowest workload associated with a significant blood lactate accumulation. Maehara et al. (21) observed a relationship between blood lactate levels and NIRS-obtained muscle deoxygenation during constant-load cycling exercise. These authors also described (during constant-load cycling exercises above the LT) an increased muscle

deoxygenation and higher blood lactate levels when O_2 delivery to muscle was presumably reduced, either by hypoxic breathing or by the inhalation of low concentrations of carbon monoxide. Thus results from the present study and from that of Maehara et al. indicate that the association between NIRS-obtained muscle deoxygenation and blood lactate levels can be described for different exercise protocols.

Although the onset of muscle deoxygenation correlated with the onset of blood lactate accumulation, as defined by a >0.5 mM change in $[La]_b$, it did not correlate with the LT defined by the 2 or 4 mM levels (1). Indeed, no significant correlation could be found between the NIRS-derived index of muscle oxygenation and the submaximal workloads associated with the fixed $[La]_b$ values conventionally employed to determine the so-called LT, i.e., 2 or 4 mM (1). In particular (see Fig. 7), the onset of muscle deoxygenation occurred at workloads that were significantly lower than those associated with $[La]_b \leq 4$ mM. Although more subjects of different training status would probably be needed to confirm such a conclusion, the results of the present study do not seem to provide a physiological basis (in terms of muscle oxygenation) for the fixed $[La]_b$ methods employed to determine the LT. It might be hypothesized, however, that the relationship observed between the 4 mM LT and sport performance levels during endurance events (34) could be related to the subjects' capacity to sustain a given level of muscle deoxygenation for relatively prolonged periods of time.

As pointed out by Belardinelli et al. (4), one of the assumptions of the present study and other similar studies is that the portion of the vastus lateralis investigated by the NIRS technique is recruited in proportion to the relative work performed. This assumption seems reasonable, considering that *1*) the placement of the probe should be over one of the motor points of the muscle (18), so that the region of the muscle should be recruited any time the muscle is activated; and *2*) according to Miyashita et al. (26), the integrated electromyogram signal from the vastus lateralis increases almost linearly with work during cycling.

The subjects of the present study were a very homogeneous group of elite altitude climbers. Although the subjects were studied a few weeks before a Himalayan expedition, they were not acclimatized to altitude at the time of the tests, as can be deduced from their blood Hb levels (see *Subjects*). Their $\dot{V}O_{2peak}$ values (see RESULTS) are compatible with those of well-trained subjects. The results of the present study, however, will have to be confirmed in future investigations conducted with subjects of different ages, training status, and gender.

Conclusions. The onset of blood lactate accumulation during incremental exercise on a cycle ergometer was highly correlated with the onset of muscle (vastus lateralis) deoxygenation, as determined by NIRS. Both events occurred at 60–65% of $\dot{V}O_{2peak}$. Because previous studies showed relatively constant femoral venous P_{O_2} levels at submaximal and maximal workloads, muscle deoxygenation observed in the present study can be attributed to capillary-venular Hb desaturation in the presence of relatively constant capillary-venular P_{O_2} levels, likely as a consequence of a rightward shift of the O_2Hb dissociation curve, determined by the onset of lactic acidosis.

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