Functional impairment of skeletal muscle oxidative metabolism during knee extension exercise after bed rest

Desy Salvadego,1 Stefano Lazzer,1 Mauro Marzorati,2 Simone Porcelli,2,3 Enrico Rejc,1 Bostjan Šimunič,4 Rado Pišot,4 Pietro Enrico di Prampero,1 and Bruno Grassi1

1Department of Medical and Biological Sciences, University of Udine, Udine; 2Institute of Bioimaging and Molecular Physiology, Consiglio Nazionale delle Ricerche, Milan; and 3Faculty of Scienze Motorie, Uni-tel San Raffaele, Rome, Italy; and 4Institute of Kinesiology Research, University of Primorska, Koper, Slovenia

Presented 24 November 2010; accepted in final form 13 September 2011

Bed rest (BR) studies are widely utilized as a model to simulate exposure to microgravity. In more general terms, BR studies allow evaluation of the consequences on physiological functions deriving from extreme degrees of physical deconditioning. An analysis of oxidative metabolism was performed in previous BR studies (see e.g., Refs. 9, 10, 19), which found significant impairments in maximal O2 uptake (V\text{O}_2\text{max}) and cardiovascular function. In these studies, however, maximal O2 extraction by skeletal muscle, as inferred from the calculated maximal systemic arterial-venous O2 concentration difference [equals V\text{O}_2\text{max}/maximum cardiac output (Q\text{c})] was unaffected by BR. This appears surprising, considering that BR induces significant morphological and biochemical changes in skeletal muscles (4, 12, 19, 20, 21, 22, 35, 37), which should affect maximal systemic arterial-venous O2 concentration difference. In a previous study by our group (38), the peak capacity of fractional O2 extraction by vastus lateralis (VL) muscle during cycle ergometer exercise was markedly reduced (by ∼30%) after 35 days of BR (2007 BR-campaign); this impairment contributed to the observed reductions in peak O2 uptake (V\text{O}_2\text{peak}) and exercise tolerance. When skeletal muscle oxidative metabolism is to be specifically evaluated, however, cycle ergometer or treadmill incremental exercise protocols are not ideal, since it is generally accepted that, during these types of exercise, the main limitation to oxidative metabolism derives from the maximal capacity of cardiovascular O2 delivery (14). This concept should apply even more strictly after BR, a condition in which, as mentioned above, cardiovascular function is substantially impaired (10, 19). In the present study, a functional evaluation of oxidative metabolism specifically aimed at skeletal muscles was carried out, before and after a 35-day BR, during a dynamic knee extension (KE) incremental exercise exercise protocol of constraints related to cardiovascular O2 delivery, a decrease in peak O2 uptake and muscle peak capacity of fractional O2 extraction was found after 35 days of BR. These findings suggest a substantial impairment of oxidative function at the muscle level, “downstream” with respect to bulk blood flow to the exercising muscles, that is possible at the level of blood flow distribution/O2 utilization inside the muscle, peripheral O2 diffusion, and intracellular oxidative metabolism.

microgravity; muscle atrophy; physical deconditioning; exercise tolerance; near-infrared spectroscopy

Materials and Methods

Experimental Design

We studied nine healthy young men (age: 23.5 ± 2.2 yr; mean ± SD; body mass: 75.0 ± 9.9 kg; height: 1.79 ± 0.07 m; body mass index: 23.4 ± 1.9 kg/m²), physically active, but without a history of high athletic achievement, who participated in the 2008 Agenzia Spaziale Italiana-Osteoporosis and Muscular Atrophy BR campaign [35-day head-down (−6°) tilt BR]. None of the subjects was affected by neuromuscular or cardiovascular disorders at the time of the study. Blood hemoglobin concentration ([Hb]) was 14.8 ± 0.9 g/dl before BR and 16.3 ± 0.8 g/dl after BR.
Participants were informed about the aims and methods of the investigation and gave their written, informed consent. The experiments were carried out at the Valdoltra Orthopaedic Hospital of Ankaran, Slovenia. All procedures conformed to the declaration of Helsinki (2000) and were approved by the Slovenian National Medical Ethics Committee.

The study consisted of three different phases: 1) The first phase was baseline control experiments before BR. 2) The second phase was a 35-day BR period without countermeasures. During BR, no deviations from the lying position were permitted, and subjects were continuously monitored by video cameras. Neither exercise nor muscle contraction tests were allowed. To avoid weight gains due to the reduced activity level, the subjects followed a balanced diet during BR. 3) The third phase was the final experiments after BR. Measurements before BR were carried out during the last day before the subjects were put to bed, whereas measurements after BR were carried out during the second day after the subjects rose from bed.

All tests were conducted under close medical supervision, and subjects were continuously monitored by 12-lead electrocardiography.

**Exercise Protocol**

An incremental exercise protocol for V\(\text{O}_2\)peak determination was carried out by utilizing a one-leg KE ergometer (modified Monark cycle ergometer), originally described by Andersen et al. (1). Subjects were constrained on an adjustable seat by a safety belt, which anchored the angle of the hip at ~90°. Subjects pushed on a padded bar attached to a lever arm, extending the leg from ~90° to ~170° flexion. This type of exercise confines muscle contractile activity to the quadriceps femoris muscle, which is involved in the leg extension phase, while the return to the starting position is brought about passively by the momentum of the fly-wheel of the ergometer. Electromyography (EMG) (see below) excluded any significant inter-vention of the hamstrings muscle group during the exercise (Fig. 1).

Before data collection, each subject was familiarized with the setup environment and the exercise protocol by short preliminary practice runs. After an initial 3 min of unloaded KE exercise, an incremental test to volitional exhaustion was performed with the right leg. Work rate increments were imposed every 2 min, to allow the subjects to test to volitional exhaustion was performed with the right leg. Work runs. After an initial 3 min of unloaded KE exercise, an incremental environment and the exercise protocol by short preliminary practice measurements before BR were carried out during the last day before the subjects were put to bed, whereas measurements after BR were carried out during the second day after the subjects rose from bed.

Net mechanical efficiency was calculated for each subject as the ratio between the external mechanical power output (work rate, expressed in Watts) and the oxidative energy output. Also, this variable was expressed in Watts by assuming an energy equivalent of 20.9 kJ/l consumed O\(_2\), and the equivalence 1 W = 1 J/s.

Heart rate (HR) was determined by electrocardiography. Stroke volume (SV) was estimated beat-by-beat by impedance cardiography (Physio Flow, Manatec, Paris, France) (40). The accuracy of this device has been previously evaluated during incremental exercise in healthy subjects against the direct Fick method (40); in that study, the correlation coefficient between the two methods was \(r = 0.946 (P < 0.01)\), the mean difference was equal to \(-2.78 \pm 12.33\% (SD 2)\), and the accuracy of the impedance cardiography method was recognized to be “acceptable”. Q was calculated as HR × SV.

Oxygenation changes in VL muscle and brain (frontal cortex) were evaluated by near-infrared spectroscopy (NIRS) (6, 15). A portable near-infrared, single-distance, continuous-wave photometer (HEO-100, Omron, Kyoto, Japan), which adopts an algorithm based on diffusion theory (43), was used for skeletal muscle measurements. The probe unit has a silicon photodiode as photodetector in the center and two light-emitting diodes (peak wavelengths 760 and 840 nm) on either side. The probe was firmly positioned to the skin overlying the lower one-third of the VL muscle (~10–12 cm above the knee joint) of the right limb, parallel to the major axis of the thigh. The sampling frequency was set at 2 Hz. The distance between each light source and the photodetector was 3 cm, corresponding to a penetration depth of ~1.5 cm. Concentration changes of oxygenated Hb + myoglobin (Mb) \(\{\Delta [\text{oxy(Hb+Mb)}]\}\) and deoxygenated Hb + Mb \(\{\Delta [\text{deoxy(Hb+Mb)}]\}\) were calculated and expressed in arbitrary units (43). The sum of the two variables \(\{\Delta [\text{oxy(Hb+Mb)}] + \text{deoxy(Hb+Mb)}]\}\) is related to changes in the total Hb volume in the muscle region of interest. \(\Delta [\text{deoxy(Hb+Mb)}]\) was taken as an estimate of skeletal muscle fractional O\(_2\) extraction because this variable, unlike \(\Delta [\text{oxy(Hb+Mb)}]\), is relatively insensitive to changes in blood volume (18, 24). A “physiological calibration” of the \(\Delta [\text{deoxy(Hb+Mb)}]\)
values was performed both before and after BR during a transient limb ischemia: data obtained during exercise were expressed as a percentage of the values determined by obtaining a maximal deoxygenation of muscle, after the exercise period, by pressure cuff inflation (at 300–350 mmHg), carried out at the inguinal crease of the thigh for a few minutes until $\Delta$(deoxy(Hb+Mb)) increase reached a plateau (for further details on the skeletal muscle NIRS measurements, see Refs. 23, 24, 30).

$\Delta$(deoxy(Hb+Mb)) kinetics were evaluated. Average data obtained during the last 20 s of each work rate were calculated and retained for analysis. As proposed by Ferreira et al. (16), the $\Delta$(deoxy(Hb+Mb)) vs. work rate relationship was fitted by a sigmoid function of the type:

$$y(x) = y_{BAS} + A/[1 + e^{-(x-c)/d}]$$  \hspace{1cm} (I)

In Eq. 1, $y_{BAS}$ indicates the baseline, $A$ is the amplitude of the response, and $c$ is a constant dependent on $d$, the slope of the sigmoid, where $c/d$ gives the x value corresponding to ($y_{BAS} + A/2$).

As for brain oxygenation, a different single-distance continuous wave NIRS instrument (Oxymon, Artinis, The Netherlands) was utilized. Headsets held a near-infrared emitter (laser light at 780 and 850 nm) and detector pair over the right frontal cortex region of the forehead; optodes were held in place by a plastic spacer with fixed odotype distance. Spacing between optodes was 4.5 cm, corresponding to a penetration depth of 2–2.5 cm. Data were recorded at 10 Hz. The Beer-Lambert law was used to calculate micromolar ($\mu$M) changes in tissue oxygenation ($\Delta$(oxyHb) and $\Delta$(deoxyHb)) by using received optical densities and a differential path-length factor of 5.93 (44). Measurements obtained during exercise were normalized as changes from an initial value arbitrarily defined as 0 $\mu$M. Total ($\Delta$(oxyHb + deoxyHb)) was taken as an index of changes in regional blood volume.

Reliability of tissue oxygenation indexes obtained by NIRS, evaluated by the intraclass correlation coefficient for repeated measurements on the same subject during different days, was recently found to be very high, for both brain and skeletal muscle (44); for brain measurements, these authors utilized the same instrument of the present study. NIRS measurements in cerebral (25) and muscle (47) tissue have been shown to be well correlated with local venous O₂ saturation. Single-site NIRS measurements, as carried out in the present study, do not allow the determination of spatial heterogeneity in the dynamics of muscle oxygenation (29). Subudhi et al. (45) recently demonstrated that brain deoxygenation during high-intensity exercise occurs across the cortex, and thus directly affects also the motor areas that regulate central motor drive. Advantages and limitations of NIRS measurements in skeletal muscle and brain tissues are discussed in the reviews by Boushel et al. (6) and Ferrari et al. (15).

EMG recordings were collected from the VL, rectus femoris (RF), and biceps femoris (BF) muscles of the exercising leg by means of a four-channel EMG system (EMG100C, BIOPAC Systems). Further technical details on the EMG measurements can be found in Rejc et al. (39). EMG raw signals, recorded during the last 10 flexion-extension cycles of each work rate, were rectified, integrated, and divided by the duration of the exercise phase considered, as to obtain a mean integrated EMG (iEMG) value.

Before KE exercise, subjects performed two maximal voluntary isometric contractions (MVC) of knee extensors with the right lower limb (knee angle at 110°). Force analog output was sampled at a frequency of 1 kHz (MP100, BIOPAC Systems). During MVC, the EMG activity was defined in a 500-ms window centered at maximal force exertion.

**Statistical Analysis**

Results were expressed as means $\pm$ SD. Statistical significance of differences between the two conditions (after vs. before BR) was checked by two-tailed Student’s $t$-test for paired data. When comparisons were made between data obtained in the present study with those obtained by Porcelli et al. (38) before and after BR during cycle ergometer exercise, a two-way repeated analysis of variance was utilized. A Tukey post hoc test was used to locate significant differences.

The level of significance was set at $P < 0.05$. Statistical analyses were carried out with a commercially available software package (Prism 4.0, GraphPad).

**RESULTS**

The main anthropometric and body composition parameters obtained before and after BR are given in Table 1. Body mass was $\sim$3% lower after BR. Percentagewise, the decrease was similar for fat-free mass, total muscle mass, and quadriceps muscle mass. Also, body fat decreased significantly after BR.

Pulmonary $V_O2$ mean ($\pm$SD) values obtained during KE before and after BR are plotted as a function of work rate in Fig. 2A. To obtain this figure, individual $V_O2$ values were grouped for discrete work rate intervals. Peak work rate was significantly lower after (45.8 $\pm$ 8.5 W) vs. before (63.2 $\pm$ 13.1 W) BR. $V_O2$ values at the same submaximal work rates were very similar before and after BR. The values increased as a function of work rate with a biphasic pattern. A steeper rise of $V_O2$ was indeed evident, starting from the work rate preceding exhaustion, at which point the slope of $V_O2$ vs. work rate increased twofold (mean of individual values went from 11.1 to 25.8 ml O₂·W$^{-1}$·min$^{-1}$ before BR and from 10.1 to 22.2 ml O₂·W$^{-1}$·min$^{-1}$ after BR). The mean net mechanical efficiency was $\sim$25% at submaximal work rates and $\sim$11% at the highest work rate, both before and after BR.

According to Richardson et al. (42), the inflection point of the $V_O2$ vs. work rate relationship during incremental KE can be attributed to the intervention of ancillary muscles to stabilize the body and to the increased cost of exercise hyperpnea. Thus, to estimate KE muscle-specific $V_O2$peak, the $V_O2$ vs. work rate linear regression obtained before the inflection point was extrapolated to peak work rate (see Fig. 2A). The estimated $V_O2$peak values are indicated by the arrows. Expressed as liters per minute, they were $\sim$19% lower after vs. before BR. When $V_O2$peak was normalized per kilogram of quadriceps muscle mass, a percentagewise similar decrease ($\sim$16%) was found (Fig. 2B). Both before and after BR, individual GET values obtained by the V-slope method (see above) corresponded approximately to the inflection point of pulmonary $V_O2$ vs. work rate; GET values were significantly lower after vs. before BR ($\sim$17%) (see Table 2). The lower $V_O2$peak and GET after BR were associated with a lower exercise tolerance, as shown by the lower time to exhaustion during the incremental KE.

| Table 1. Anthropometric characteristics of subjects |
|-----------------------------------|-----------------------------------|----------------|
| **Before BR** | **After BR** | **Change, %** |
| Body mass, kg | 75.0 ± 9.9 | 72.4 ± 9.2* | -3.4 |
| BML, kg/m² | 23.4 ± 1.9 | 22.6 ± 1.7* | -3.4 |
| FFM, kg | 62.1 ± 6.3 | 60.6 ± 5.8* | -2.4 |
| Total muscle mass, kg | 31.9 ± 3.7 | 30.9 ± 3.4* | -3.1 |
| Quadriceps muscle mass, kg | 2.21 ± 0.16 | 2.13 ± 0.14* | -3.8 |
| Body fat, % | 17.0 ± 3.6 | 16.0 ± 3.2* | -5.1 |

Values are means $\pm$ SD for anthropometric and body composition data before and after bed rest (BR). Mean percentage change for each parameter is also reported. BML, body mass index; FFM, fat-free mass. *Significantly different vs. values before BR ($P < 0.05$).
exercise (9.8 ± 2.1 min after vs. 12.3 ± 3.4 min before BR). Respiratory and cardiovascular variables at exhaustion were not affected by BR (Table 2).

NIRS-obtained brain oxygenation data during KE are shown in Fig. 3. Mean (±SD) values of individual Δ[oxyHb], Δ[deoxyHb], and Δ[oxyHb+deoxyHb] data, grouped for discrete work rate intervals, are reported as a function of work rate. Both before and after BR, Δ[deoxyHb] values did not change significantly from the baseline, at any work rate. The Δ[oxyHb] did not increase from the baseline at the first work rate, after which it increased markedly up to exhaustion. Values after BR were significantly lower, both at submaximal (30 W) and at maximal work rates. The relationship between Δ[oxyHb+deoxyHb] and work rate was very similar to that of Δ[oxyHb].

Mean (±SD) values of the NIRS-obtained muscle oxygenation index, Δ[deoxy(Hb+Mb)], which was taken as an estimate of VL fractional O2 extraction, are shown as a function of work rate in Fig. 4. Before BR, Δ[deoxy(Hb+Mb)] increased following a sigmoid pattern (Eq. 1), approaching a plateau at ~75% of peak work rate. After BR, Δ[deoxy(Hb+Mb)] was significantly higher than before BR at the lowest work rate, whereas the ensuing Δ[deoxy(Hb+Mb)] increase was much less pronounced than before BR. The Δ[deoxy(Hb+Mb)] peak values were significantly lower after vs. before BR.

iEMG values (mV) for VL, RF, and BF during KE are shown in Fig. 5. In VL and RF, iEMG values increased as a function of work rate. Peak iEMG values were lower after vs. before BR, in association with the lower peak work rate. For the same absolute work rate, iEMG values were not different after vs. before BR, except for RF at 30 W (significantly higher values after BR). Increased iEMG is a sign of higher muscle activation, presumably attributable to recruitment of additional motor units. iEMG values for BF did not change substantially from the baseline, confirming that the knee flexors were not significantly activated during the exercise protocol (see also Fig. 1). The mean force exerted by the knee extensors during MVC was significantly lower after (530.1 ± 111.9 N) vs. before (683.2 ± 95.7 N) BR. The decrease in force was associated with iEMG values during MVC that were lower after (0.225 ± 0.076 mV for VL and 0.376 ± 0.199 mV for RF) vs. before (0.277 ± 0.05 mV for VL and 0.501 ± 0.237 mV for RF) BR.

**DISCUSSION**

In this study, an incremental KE exercise was utilized to investigate skeletal muscle oxidative function in a group of young physically active volunteers undergoing 35 days of BR. During KE, the recruitment of a relatively small muscle mass, i.e., the quadriceps femoris of one leg, significantly reduces or eliminates any constraint to oxidative function deriving from cardiovascular O2 delivery, thereby highlighting any impairment intrinsic to skeletal muscle. As the main findings of the study, we observed after BR (vs. before) the following: 1) a significant decrease in V˙O2peak, GET, and time to exhaustion during the incremental KE exercise; 2) no change in peak cardiovascular O2 delivery, which reached levels well below those usually found during cycle ergometer exercise; 3) a preserved cerebral oxygenation; and 4) a significant decrease in the peak capacity of fractional O2 extraction by VL muscle. These findings suggest a relevant impairment of oxidative function occurring during BR at the skeletal muscle level. This impairment is a component of the microgravity-induced muscle deterioration and has a negative impact on exercise tolerance.

Before the “inflection point” (see Fig. 2), presumably attributable to the intervention of ancillary muscles to stabilize the

<table>
<thead>
<tr>
<th>Table 2. Peak oxygen uptake and related parameters before and after bed rest</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before BR</th>
<th>After BR</th>
<th>Change, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>V˙E peak, l/min</td>
<td>45.7 ± 6.9</td>
<td>41.4 ± 8.8</td>
<td>-8.0</td>
</tr>
<tr>
<td>V˙T peak, liter</td>
<td>1.32 ± 0.25</td>
<td>1.29 ± 0.17</td>
<td>0.0</td>
</tr>
<tr>
<td>IV peak, breaths/min</td>
<td>35.5 ± 5.1</td>
<td>32.3 ± 5.1</td>
<td>-8.0</td>
</tr>
<tr>
<td>V˙O2peak, l/min</td>
<td>1.26 ± 0.27</td>
<td>0.99 ± 0.17*</td>
<td>-19.1</td>
</tr>
<tr>
<td>V˙O2peak/QM, ml·min⁻¹·100 g⁻¹</td>
<td>56.9 ± 11.0</td>
<td>46.5 ± 6.4*</td>
<td>-16.2</td>
</tr>
<tr>
<td>Rpeak, l/min</td>
<td>1.08 ± 0.08</td>
<td>1.09 ± 0.10</td>
<td>+1.7</td>
</tr>
<tr>
<td>GET, l/min</td>
<td>1.02 ± 0.15</td>
<td>0.84 ± 0.15*</td>
<td>-16.9</td>
</tr>
<tr>
<td>Time to exhaustion, min</td>
<td>12.3 ± 3.4</td>
<td>9.8 ± 2.1*</td>
<td>-18.3</td>
</tr>
<tr>
<td>HRpeak, beats/min</td>
<td>147 ± 18</td>
<td>146 ± 17</td>
<td>-0.3</td>
</tr>
<tr>
<td>SVpeak, ml</td>
<td>122 ± 25</td>
<td>109 ± 16</td>
<td>-7.9</td>
</tr>
<tr>
<td>Qpeak, l/min</td>
<td>17.8 ± 3.3</td>
<td>16.1 ± 1.8</td>
<td>-7.0</td>
</tr>
</tbody>
</table>

Values are means ± SD. Values are obtained during incremental knee extension exercise at exhaustion (peak values) before and after BR. V˙E, pulmonary ventilation; V˙T, tidal volume; IV, ventilatory frequency; V˙O2peak, peak oxygen uptake; V˙O2peak/QM, oxygen uptake normalized per unit of quadriceps muscle mass; Rpeak, peak gas exchange ratio; GET, gas exchange threshold: time to exhaustion, exercise duration sustained by the subjects; HRpeak, peak heart rate; SVpeak, peak stroke volume; Qpeak, peak cardiac output. *Significantly different from the corresponding value before BR (P < 0.05).

**J Appl Physiol • VOL 111 • DECEMBER 2011 • www.jap.org**
by Bringard et al. (7), who observed lower V\textsubscript{O2}\text{peak} values after impairment of oxidative metabolism at peak exercise observed elimination of cardiovascular constraints did not influence the period, but during cycle ergometer exercise (19, 38). Thus similar to that observed in previous studies after the same BR function of work rate during KE. The was utilized to estimate VL fractional O\textsubscript{2} extraction, are shown as a function of work rate. *P < 0.05, before vs. after BR at the same relative work rate. See text for further details.

On the other hand, V\textsubscript{O2}\text{peak} values were significantly lower after BR. Percentagewise (~19%), the reduction was very similar to that observed in previous studies after the same BR period, but during cycle ergometer exercise (19, 38). Thus elimination of cardiovascular constraints did not influence the impairment of oxidative metabolism at peak exercise observed after BR. This appears in agreement with recent data obtained by Bringard et al. (7), who observed lower V\textsubscript{O2}\text{peak} values after BR in subjects cycling in the supine posture, which allowed V\textsubscript{O2}\text{peak} to be substantially unaffected by BR.

The extent of decrease in V\textsubscript{O2}\text{peak} did not differ substantially (~16%) once the BR-induced reduction in specific muscle mass was taken into account, and it was also very similar to that observed for GET (~17%). Lower values of V\textsubscript{O2}\text{peak} and GET after BR were associated with (and presumably were responsible for) a significant impairment of exercise tolerance, as shown by the significantly lower peak work rate and time to exhaustion values.

Peak cardiovascular O\textsubscript{2} delivery was unaffected by BR. A small decrease in V\textsubscript{O2}\text{peak} after BR was indeed offset by a small increase in [Hb]. Peak cardiovascular O\textsubscript{2} delivery, estimated as the product of V\textsubscript{O2}\text{peak} and arterial O\textsubscript{2} concentration (calculated on the basis of the measured [Hb], after assuming an arterial H\textsubscript{b} saturation of 98% and an O\textsubscript{2} binding coefficient for H\textsubscript{b} of 1.34 ml/g, and after neglecting the small contribution of physically dissolved O\textsubscript{2}), was 3.51 l O\textsubscript{2}/min before and 3.43 l O\textsubscript{2}/min after BR. This scenario is different from the substantial decrease in V\textsubscript{O2}\text{peak} after BR observed by previous authors during cycling exercise (9, 10, 19, 22, 38). In the present study, cardiovascular function at exhaustion, as represented by V\textsubscript{O2}\text{peak} values, was exploited for ~70–75% of the peak levels reached during incremental cycle ergometer exercise, carried out before and after the same BR period of the present study [Porcelli et al. (38)]. This indirectly confirms that KE exercise, by involving only relatively small muscle masses (on average, 2.1–2.2 kg in the present study), did not represent a significant burden for the cardiovascular system.

Brain (frontal cortex) oxygenation, as estimated by NIRS, increased with work rate during KE, reaching the highest values at maximal exertion. This is suggested by the increased oxy signal (Δ[oxyH\textsubscript{b}]), by the unchanged deoxy signal (Δ[deoxyH\textsubscript{b}]), and by the increased total H\textsubscript{b} signal (Δ[oxyH\textsubscript{b}+deoxyH\textsubscript{b}]). These patterns were unaffected by BR. The data suggest vasodilatation in cerebral tissue, mainly represented by oxygenated blood. Thus they allow exclusion of any increased O\textsubscript{2} extraction or tissue hypoxia, possibly limiting exercise tolerance, as it has been described during exhausting cycle ergometer exercise (36). The results of the present study are not surprising, considering the relatively small muscle mass involved in KE and the consequent lack of cardiovascular constraints.

NIRS-obtained muscle Δ[deoxy(H\textsubscript{b}+Mb)] has been frequently utilized to estimate fractional O\textsubscript{2} delivery (the ratio between O\textsubscript{2} utilization and O\textsubscript{2} delivery) in skeletal muscle (11, 16, 23, 24, 30, 38). The dynamics of Δ[deoxy(H\textsubscript{b}+Mb)] as a function of work rate during incremental cycle ergometer exercise have been described by Ferreira et al. (16) by fitting a sigmoid function. A similar pattern was observed in the present study for the data obtained before BR. After BR, on the other

---

**Fig. 3.** Near-infrared spectroscopy (NIRS)-obtained brain (frontal cortex) oxygenation data during KE before and after BR. Mean (SD) values of changes in concentration of oxyhemoglobin (Δ[oxyH\textsubscript{b}]: A), changes in concentration of deoxyhemoglobin (Δ[deoxyH\textsubscript{b}]: B), and changes in concentration of total (oxy + deoxy) hemoglobin (Δ[oxyH\textsubscript{b}+deoxyH\textsubscript{b}]: C), all expressed as micromolar changes relative to an initial value arbitrarily set equal to zero, are shown as a function of work rate. *P < 0.05, before vs. after BR at the same relative work rate. See text for further details.

**Fig. 4.** Mean (SD) values of the NIRS-obtained muscle oxygenation index [concentration changes of deoxygenated H\textsubscript{b} + myoglobin (Mb); Δ[deoxy(H\textsubscript{b}+Mb)]] with % of ischemia, which was utilized to estimate VL fractional O\textsubscript{2} extraction, are shown as a function of work rate during KE. The Δ[deoxy(H\textsubscript{b}+Mb)] data are expressed as a percentage of those obtained during a transient limb ischemia induced at the end of the test. The sigmoid function (Eq. 1) utilized to fit the data before BR is also shown. *P < 0.05 vs. before BR data. See text for further details.
hand, $\Delta[\text{deoxy(Hb+Mb)}]$ values were higher than before BR at the lowest work rate and increased only slightly at higher work rates. Higher $\Delta[\text{deoxy(Hb+Mb)}]$ for the same work rate, and for the same $\dot{V}\text{O}_2$ (efficiency of KE was unaffected by BR, see above), would suggest an enhanced tissue $\text{O}_2$ extraction to sustain the needed $\dot{V}\text{O}_2$ (30), assuming an identical optode placement and muscle penetration depth. Increased muscle fractional $\text{O}_2$ extraction at submaximal work rates has been observed during KE in acute hypoxic exposure (41); in that study, the increased fractional $\text{O}_2$ extraction compensated for the reduction in $\text{O}_2$ delivery. In that study, moreover, fractional $\text{O}_2$ extraction rose rapidly with work rate, attaining a plateau at values matching those found in normoxic condition. This was not the case for the present study after BR: $\Delta[\text{deoxy(Hb+Mb)}]$ increased only slightly as a function of work rate, reaching peak values (~45% of the maximal values obtained by a transient ischemia), which were significantly lower than those obtained before BR (~65%). The impairment in peak fractional $\text{O}_2$ extraction observed in the present study after BR is very similar to that observed, after a similar BR exposure, by Porcelli et al. (38) during incremental cycle ergometer exercise. Thus, as also discussed above for $\dot{V}\text{O}_2\text{peak}$, with the present exercise protocol, in which constraints imposed by $\text{O}_2$ delivery were minimized, a BR-induced impairment in peak capacity for muscle fractional $\text{O}_2$ extraction was still evident.

In normal subjects, in normoxia and during whole body exercise, $\dot{V}\text{O}_2\text{max}$ is often considered to be mainly limited by the maximal capacity of $\text{O}_2$ delivery to the exercising muscles (13, 14, 31). As discussed above, cardiovascular function (maximum $Q$) during incremental cycle ergometer exercise is significantly affected by BR (9, 19, 22, 38). However, as also mentioned above, in the present study, the KE exercise protocol eliminated the constraints to $\dot{V}\text{O}_2\text{max}$ related to cardiovascular $\text{O}_2$ delivery, which, moreover, was unaffected by BR. It should be noted that the studies that examined muscle blood flow during KE [see e.g., Richardson et al. (41)] measured it in the vein draining from the muscles. To the best of our knowledge, the spatial distribution of blood in the muscle has never been determined during KE. Moreover, the distribution of blood flow should be evaluated in relation to the distribution of metabolism. The ratio between $\text{O}_2$ utilization and $\text{O}_2$ delivery is reflected by the $\Delta[\text{deoxy(Hb+Mb)}]$ signal obtained by NIRS. Koga et al. (29) recently described by multichannel NIRS a significant heterogeneity of $\Delta[\text{deoxy(Hb+Mb)}]$ kinetics in the quadriceps muscle during constant-load cycle ergometer exercise. The authors did not report if this heterogeneity was present also at steady state. We do not know of similar studies carried out during KE exercise or after BR. Maximal fractional $\text{O}_2$ extraction during KE, however, was found to be very similar to that obtained during cycle ergometer exercise (38). Thus it may be assumed that the relationship between distribution of blood flow/distribution of metabolism is substantially unaffected by the high blood flows observed during KE. We recognize, however, that this relationship could, in theory, be altered by BR. At the same submaximal work rate and pulmonary $\dot{V}\text{O}_2$, the higher $\text{O}_2$ extraction estimated by NIRS after BR (see Fig. 4) would suggest an overall impairment of $\text{O}_2$ delivery. Any alteration of $\text{O}_2$ delivery and/or of the $\text{O}_2$ delivery/$\dot{V}\text{O}_2$ utilization distribution [the latter variable would belong to the “peripheral” factors ($\text{FP}$) in the model by di Prampero (see below)] could impair oxidative metabolism independently from intramyocyte function.

The multifactorial model of $\dot{V}\text{O}_2\text{max}$ limitation proposed by di Prampero (for details, see Refs. 13, 14) was applied to the present KE data as an attempt to quantify the contribution of “central” factors ($\text{FQ}$) and $\text{FP}$ in setting the upper limit to oxidative metabolism after BR. $\text{FQ}$ included peripheral vascular $\text{O}_2$ delivery, peripheral $\text{O}_2$ diffusion, and intramyocyte factors [represented here by the NIRS-obtained $\Delta[\text{deoxy(Hb+Mb)}]$ in the VL muscle]. $\text{FP}$ included cardiovascular $\text{O}_2$ transport [represented here by the product of $Q\text{peak}$ times [Hb]]. $\text{FP}$ and $\text{FQ}$ amounted to 0.56 and to 0.44, respectively, supporting the notion that $\text{FP}$ are the main determinant of $\dot{V}\text{O}_2\text{peak}$ during normoxic exercise with small muscle mass. It may be of interest to observe that, upon the data described above, the reduction in $\dot{V}\text{O}_2\text{peak}$ following BR observed in the present study can be fully explained by the impairment in the peak capacity of fractional $\text{O}_2$ extraction by skeletal muscles. By accounting for ~60% in limiting $\dot{V}\text{O}_2\text{peak}$ during KE, the observed ~30% decrease in fractional $\text{O}_2$ extraction led to a $\dot{V}\text{O}_2\text{peak}$ decrease of ~18–20%.

Our findings are similar to those obtained by McGuire et al. (34) in aged subjects; in both studies, a profound physical deconditioning, either BR or age related, determined a decline in oxidative function, which involved primarily the skeletal muscles.

Impairments of peripheral $\text{O}_2$ diffusion and/or $\text{O}_2$ utilization by muscle fibers could be attributable to structural and functional changes, which may involve both slow- and fast-twitch fibers, such as decreases in volume density of mitochondria,
muscle oxidative enzymes activity, muscle capillary length and density (4, 19, 26); muscle fiber atrophy, greater in postural muscle groups and in slow type I vs. fast type II fibers (5, 20, 21); and substantial downregulation of proteins involved in oxidative metabolism (35). Moreover, qualitative changes, such as a shift in the contractile and metabolic phenotype toward that of a fast-twitch muscle, with significant reductions in myosin heavy chain-1 and increases in myosin heavy chain-2X relative content, and changes in motor unit recruitment profiles (12) toward a preferential recruitment of fast-twitch fibers, have been described after BR (5, 8, 27). It is noteworthy that changes in muscle oxidative capacity, vascular control, and fiber-type composition and/or recruitment may be associated with alterations in the O2 delivery to O2 utilization relationship. Previous studies, for instance, measured higher O2 extractions and lower microvascular O2 pressures, at low metabolic rates, in fast-twitch muscles, or in muscle regions in which these fibers predominate, compared with slow-twitch muscles (3, 17, 33).

Conclusions

We recognize that the quantitative analysis of the upper limits to oxidative metabolism (13, 14) described above may represent an oversimplification, since “central” and “peripheral” constraints to VO2 max are closely interrelated, as demonstrated by Peter Wagner’s group over the years [see, e.g., Wagner (46)]. Our reasoning, however, should be straightforward. By utilizing the KE exercise protocol (relatively small muscle mass), 1) peak cardiovascular O2 delivery was not affected by BR; and 2) on the other hand, VO2peak and peak fractional O2 extraction in the exercising muscles were substantially lower after BR. Thus these decreases should be attributable to factors “downstream” with respect to bulk blood flow to the exercising muscles that is at the level of blood flow distribution/O2 utilization inside the muscle, peripheral O2 diffusion, and intracellular oxidative metabolism. Besides being relevant from the point of view of microgravity-induced muscle deterioration and for the definition of countermeasures aimed at reversing it, the present results appear of interest also for rehabilitation purposes following extreme physical deconditioning.

ACKNOWLEDGMENTS

The authors are grateful to Gianni Biolo and Dr. Francesco Agostini (University of Trieste, Italy) for the excellent organization and coordination of the Valdoltra 2008 BR campaign, to the staff of the Valdoltra Hospital for the excellent technical collaboration and supervision of the subjects during the BR, and to the volunteers of the study for kind determination to perform our experiments. The authors also thank Ranieri Barelli and Luca Bazzichetto, whose technical assistance during the implementation of the KE ergometer was invaluable.

GRANTS

Financial support by the Agenzia Spaziale Italiana (ASI-OSMA Contract I007/06/03-Workpackage 1B-32-1) and by Telethon-UILDM Italy (project GUP08007) is acknowledged.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: D.S., S.L., M.M., S.P., E.R., and B.S. performed experiments; D.S., S.L., M.M., S.P., E.R., and B.S. analyzed data; D.S., R.P., P.E.D., and B.G. interpreted results of experiments; D.S. prepared figures; D.S. and B.G. drafted manuscript; D.S., P.E.D., and B.G. edited and revised manuscript; D.S. and B.G. approved final version of manuscript; B.G. conception and design of research.

REFERENCES


24. **Grassi** B, **Pogliaghi** S, **Rampichini** S, **Quaresima** V, **Ferrari** M, **Marconi** C, **Cerretelli** P. Muscle oxygenation and pulmonary gas exchange kinetics during cycling exercise in transition on humans. *J Appl Physiol* 95: 149–158, 2003.

25. **Henson** LC, **Calalang** C, **Temp** JA, **Ward** DS. Accuracy of a cerebral oximeter in healthy volunteers under conditions of isocapnic hypoxia. *Anesthesiology* 88: 58–65, 1998.


29. **Koga** S, **Poole** DC, **Ferreira** LF, **Whipp** BJ, **Kondo** N, **Saitoh** T, **Ohmae** E, **Barstow** TJ. Spatial heterogeneity of quadriceps muscle deoxygenation kinetics during cycle exercise. *J Appl Physiol* 103: 2049–2056, 2007.

30. **Lanfranconi** F, **Borrelli** E, **Porcelli** S, **Maccherini** M, **Chiaurelli** M, **Grassi** B. Noninvasive evaluation of skeletal muscle oxidative metabolism after heart transplant. *Med Sci Sports Exerc* 38: 1374–1383, 2006.


32. **Lukaski** HC, **Bolonchuk** WW, **Hall** CB, **Siders** WA. Validation of tetrapolar bioelectrical measurements to assess human body composition. *J Appl Physiol* 60: 1327–1332, 1986.

33. **McDonough** P, **Behnke** BJ, **Padilla** DJ, **Musch** TI, **Poole** DC. Control of microvascular oxygen pressures in rat muscles comprised of different fibre types. *J Physiol* 563: 903–913, 2005.

34. **McGuire** DK, **Levine** BD, **Williamson** JW, **Snell** PG, **Blomqvist** CG, **Saltin** B, **Mitchell** JH. A 30-year follow-up of the Dallas Bedrest and Training Study. II. Effect of age on cardiovascular adaptation to exercise training. *Circulation* 104: 1358–1366, 2001.

35. **Moriggio** M, **Vasso** M, **Fania** C, **Capitanio** D, **Bonifacio** G, **Salanova** M, **Bottner** D, **Rittweger** J, **Felsenberg** D, **Cerretelli** P, **Gelli** C. Long term bed rest with and without vibration exercise countermeasures: effects in human muscle protein dysregulation. *Proteomics* 10: 3758–3774, 2010.


40. **Richard** R, **Lonsdorfer-Wolf** E, **Charloux** A, **Doutrileau** S, **Bucheit** M, **Oswald-Mammosser** M, **Lampert** E, **Mettauer** B, **Geny** B, **Lonsdorfer** J. Non-invasive cardiac output evaluation during a maximal progressive exercise test, using a new impedance cardiograph device. *Eur J Appl Physiol* 85: 202–207, 2001.

41. **Richardson** RS, **Knight** DR, **Poole** DC, **Kurdak** SS, **Hogan** MC, **Grassi** B, **Wagner** PD. Determinants of maximal exercise V̇O₂ during single leg knee-extensor exercise in humans. *Am J Physiol Heart Circ Physiol* 268: H1453–H1461, 1995.

42. **Richardson** RS, **Poole** DC, **Knight** DR, **Kurdak** SS, **Hogan** MC, **Grassi** B, **Johnson** EC, **Kendrick** KF, **Erickson** BK, **Wagner** PD. High muscle blood flow in man: is maximal O₂ extraction compromised? *J Appl Physiol* 75: 1911–1916, 1993.

43. **Shiga** T, **Yamamoto** K, **Tanabe** K, **Nakase** Y, **Chance** B. Study of an algorithm based on model experiments and diffusion theory for a portable tissue oximeter. *J Biomed Opt* 2: 154–161, 1997.


47. **Wilson** JR, **Mancini** DM, **McCully** K, **Ferraro** N, **Lanoce** V, **Chance** B. Noninvasive detection of skeletal muscle underperfusion with near-infrared spectroscopy in patients with heart failure. *Circulation* 80: 1668–1674, 1989.