Peripheral impairments of oxidative metabolism after a 10-day bed rest are upstream of mitochondrial respiration

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Abstract In order to identify peripheral biomarkers of impaired oxidative metabolism during exercise following a 10-day bed rest, 10 males performed an incremental exercise (to determine peak pulmonary $\dot{V}O_2 (\dot{V}O_2p)$) and moderate-intensity exercises, before (PRE) and after (POST) bed rest. Blood flow response was evaluated in the common femoral artery by Eco-Doppler during 1 min of passive leg movements (PLM). The intramuscular matching between $O_2$ delivery and...
Introduction

It is well established that prolonged inactivity negatively affects almost all physiological systems (Booth et al. 2017). Bed rest studies offer a unique opportunity to evaluate the effects of prolonged muscle disuse and unloading, conditions experienced by bedridden patients with injuries and chronic diseases or by astronauts exposed to microgravity during spaceflight missions (Pavy-Le Traon et al. 2007). Exposure to microgravity or physical inactivity leads to impairment of oxidative metabolism, the main energy source for exercise or work activities lasting longer than 1–2 min. The impairment of oxidative metabolism during exercise is usually identified and quantified in terms of decreases in maximal or peak O$_2$ consumption (V'O$_2$peak) (Ried-Larsen et al. 2017). However, the sites or functions responsible for this impairment are still debated. Whereas cardiovascular impairments, mainly represented by a decreased peak cardiac output ($\dot{Q}_{\text{peak}}$) have been well described (see, e.g. Saltin et al. 1968; Ferretti et al. 1997; Porcelli et al. 2010), peripheral (microvascular and mitochondrial) impairments appear to be more complex and somehow less defined.

Capelli et al. (2006) and Ferretti et al. (2009) utilized the multifactorial model of V'O$_2$max limitation originally developed by di Prampero and Ferretti (1990), and concluded that the fractional limitation imposed by peripheral factors was about 30% after 42 days of bed rest, and about 40% after 90 days. More recent investigations reinforced the concept of peripheral factors as contributors to the decreased V'O$_2$peak following microgravity exposure (Ade et al. 2015, 2017). Ade et al. (2015) utilized a two-component model originally proposed by Wagner et al. (1993), characterized by convective O$_2$ transport to the skeletal muscle capillary bed and by the diffusive O$_2$ transport from capillaries to mitochondria, and concluded that the decline in V'O$_2$peak is initially (the first 10 days) mainly caused by a decrease in convective O$_2$ transport, whereas the role of diffusive O$_2$ transport increases with longer exposures. Salvadego et al. (2016, 2018) confirmed the presence of a peripheral impairment
of oxidative metabolism following bed rest by utilizing an experimental approach (dynamic knee extension of one limb) in which the reduced muscle mass significantly limits cardiovascular constraints (Richardson et al. 1993).

In terms of peripheral impairments, however, the respective roles of microvascular/endothelial function and mitochondrial respiration have not been systemically investigated. Porcelli et al. (2010) identified, following 35 days of bed rest, indirect signs of a mismatch between intramuscular O₂ delivery and O₂ uptake. The effects of shorter periods of bed rest on maximal mitochondrial respiration ex vivo are controversial. While Miotto et al. (2019) and Dirks et al. (2020) described an impaired mitochondrial function following bed rest periods of 3 and 7 days, respectively, other authors (Salvadego et al. 2016; Larsen et al. 2018) did not see impairments following 4 and 10 days of bed rest exposure.

The general aim of the present study was to identify peripheral biomarkers of impairment of oxidative metabolism following a relatively short (10 days) exposure to horizontal bed rest, with specific attention toward the respective roles of microvascular/endothelial function and mitochondrial respiration. More specifically, we investigated microvascular/endothelial function by utilizing the passive leg movement (PLM) approach recently described by Gifford & Richardson (2017). By utilizing near-infrared spectroscopy (NIRS) (Grassi et al. 2016; Quresima, Barstow 2019) we also looked for signs of impaired intramuscular matching between O₂ delivery and O₂ uptake (Porcelli et al. 2010, 2014, 2016; Grassi et al. 2019). We assessed mitochondrial function by utilizing two approaches, one in vivo (muscle ˙V̇O₂ recovery kinetics by NIRS and the repeated short occlusions method (Ryan et al. 2012; Adami & Rossiter, 2018; Zuccarelli et al. 2020)), and one ex vivo (mitochondrial respiration by high-resolution respirometry on permeabilized skeletal muscle fibres obtained by biopsy (Pesta & Gneiger, 2012)). From the analysis of the above-mentioned functional biomarkers we will be able to discriminate whether the peripheral limitations to oxidative metabolism are mainly upstream or at the level of mitochondrial function.

### Methods

#### Ethical approval

This study was part of the Italian Space Agency (ASI) project ‘MARS-PRE Bed Rest SBI 2019’. It was approved by the National Ethical Committee of the Slovenian Ministry of Health (ref. number: 0120-304/2019/9) and was performed in accordance with the standard set by the Declaration of Helsinki. All participants were informed about the aims, procedures and potential risks of the investigations before written consent was obtained.

### Subjects

Ten young, healthy, recreationally active males (age, 23 ± 5 yr; height, 1.81 ± 0.04 m; body mass (BM), 77.5 ± 10.0 kg; body mass index (BMI), 23.5 ± 2.5 kg m⁻²) participated in this study. Body mass composition was estimated in PRE and POST from data obtained by bioelectrical impedance analysis (Maltron 916s, UK). Participants’ characteristics at baseline are given in Table 1. Subjects underwent a medical screening before the study. Exclusion criteria were: regular smoking, habitual use of drugs, blood clotting defects, history of deep vein thrombosis with D-dimer values >500 μg L⁻¹; acute or chronic skeletal, neuromuscular, metabolic and cardiovascular disease conditions, previous history of embolism, inflammatory diseases, psychiatric disorders, epilepsy, participation in sports at a competitive level and presence of ferromagnetic implants.

### Experimental protocol

Each subject was evaluated before (PRE) and after (POST) 10 days of strict horizontal bed rest without countermeasures. The experiments were carried out at the Izola General Hospital, Slovenia. Participants arrived at the hospital 3 days before bed rest, and immediately after the PRE measurements were finished they entered the bed rest. POST measurements were carried out during the first 2 days after subjects arose from the bed. During bed rest no deviations from the lying position, muscle stretching or static contraction were allowed. Adherence to the assigned protocol was ensured using continuous closed-circuit television surveillance and constant supervision by researchers and medical staff. Subjects consumed an individually controlled, standardized diet and were allowed to drink water ad libitum. The dietary energy requirement was designed for each subject by multiplying resting energy expenditure (calculated by using the FAO/WHO equation and fat-free mass and fat mass data obtained by bioelectrical impedance (Müller et al. 2004)) by factors 1.2 and 1.4 in the bed rest and ambulatory periods, respectively (Biolo et al. 2008).
macronutrient food content was set at 60% carbohydrates, 25% fats and 15% proteins.

The environmental conditions within the hospital remained stable throughout the experimental sessions. Before data collection, subjects were familiarized with the investigators, experimental arrangements and with the exercise protocols by means of short preliminary practice runs.

Both at PRE and POST the same schedule was followed. During baseline data collection day 1 (BDC1) subjects performed a PLM experimental session (see details below) for the evaluation of peripheral vascular and endothelial functions. PLM was followed by an incremental exercise (INCR) up to voluntary exhaustion on a cycle ergometer, for the determination of pulmonary VO$_2$peak. During baseline data collection day 2 (BDC2) two repetitions of constant work rate cycling exercises of moderate intensity were performed. NIRS-obtained muscle oxygenation data at the onset of the constant work rate exercise were analysed in terms of the ‘overshoot’ of muscle deoxygenation, an indirect sign of a work rate increment of moderate intensity were performed. NIRS-obtained muscle oxygenation data at the onset of the constant work rate exercise were analysed in terms of the ‘overshoot’ of muscle deoxygenation, an indirect sign of a work rate increase.

Measurements obtained during the two repetitions were superimposed and average values were obtained for each subject and retained for data analysis. Resting blood flow and peak blood flow during PLM were calculated. The area under the blood flow vs. time response (area under the curve, AUC) was obtained by calculating the integral of the function over the entire 60 s, after subtracting the resting baseline value (see Gifford & Richardson, 2017).

Intramuscular matching between O$_2$ delivery and O$_2$ utilization

Oxygenation changes in four different sites of the anterior compartment (vastus lateralis and rectus femoris muscles) of the right thigh were determined by a portable continuous-wave NIRS instrument (OctaMon M; Artinis Medical System, The Netherlands). In the present study only the data related to the probe positioned on the lower third of the vastus lateralis muscle are presented. The skin overlying the investigated muscle region was carefully shaven before the experimentation, and the place where the probe was attached was recorded using a skin marker; this allowed the aposition of the probe in the same position during all tests. Adipose tissue thickness (ATT) at the site of application of the NIR probe was determined by a caliper (Gima, Milan, Italy). Black bandages were put around the probe and the skin to prevent contamination from ambient light. The sampling frequency was set at 10 Hz. The light transmitter...
V\textsubscript{O2}m was estimated by calculating the slope of the initial (3 s) linear increase in NIRS-measured Δ[deoxy(Hb + Mb)] during short (5 s) bouts of ischaemia induced by rapid (less than 1 s) inflation and deflation of a pneumatic cuff (∼300 mmHg) (Hokanson E20 cuff inflator, Bellevue, WA, USA) during the recovery from moderate-intensity constant work rate exercise (Zuccarelli et al. 2020). When muscle reached a deoxygenation target of 50% of the physiological normalization (Adami et al. 2017) (see above), several intermittent arterial occlusions were performed: the first five occlusions lasted 5 s each and were separated by 5 s; they were followed by five occlusions lasting 5 s each and separated by 10 s, and finally by five occlusions lasting 5 s and separated by 20 s. When the target 50% was not reached at the end of the exercise protocol the first arterial occlusion was performed after 5–10 s.

V\textsubscript{O2}m values were then fitted by a monoexponential function according to eqn (2) (Ryan et al. 2014):

\[ y(t) = y_{\text{END}} - \Delta \times e^{-t/\tau} \]  

where \( y(t) \) represents the value of \( \dot{V}\textsubscript{O2}m \) at a given time \( t \), \( y_{\text{END}} \) the \( \dot{V}\textsubscript{O2}m \) immediately after the cessation of the exercise, \( \Delta \) is the change in \( \dot{V}\textsubscript{O2}m \) from rest to end exercise and \( \tau \) is the rate constant (\( k = 1/\tau \), expressed in min\(^{-1}\)) of the function.

Resting \( \dot{V}\textsubscript{O2}m \) was estimated by calculating a linear regression of the \( \Delta[\text{deoxy(Hb + Mb)]} \) increases during the first 60 s of the physiological normalization procedure (see above). Also in this case the NIRS signals during ischaemia were normalized for changes in blood volume by utilizing the method proposed by Ryan et al. (2012).

Mitochondrial respiration \textit{ex vivo}

Skeletal muscle biopsies were obtained from the vastus lateralis muscle under local anaesthesia (2% lidocaine). The biopsy was taken immediately before and at the end of the bed rest intervention. Following the application of the anaesthetic, a 1.0–1.5 cm incision was made to the skin, subcutaneous tissue and muscle fascia, and the tissue sample was harvested with a Rongeur-Conchotome (GmbH&Co, Zepf Instruments, Dürbheim, Germany). The collected muscle tissue was dissected free of fat and connective tissue and rapidly divided into several portions. A small portion (2.0–6.5 mg wet weight) was used immediately to evaluate mitochondrial respiration \textit{ex vivo} (Pesta & Gnaiger 2012). Measurements were performed in duplicate. The portion of tissue was immediately placed in an ice-cold preservation solution (BIOOPS; Oroboros Instruments, Innsbruck, Austria) (4°C) containing: EGTA-calcium buffer (10 mM) (free Ca\(^{2+}\) concentration 100 nmol L\(^{-1}\)), imidazole (20 mM), taurine (20 mM), K+/4 morpholinoethanesulphonic acid (MES) (20 mM), Mg\(^{2+}\) (2 mM), KCl (130 mM), pH 7.4. Skeletal muscle biopsies were obtained from the vastus lateralis muscle under local anaesthesia (2% lidocaine). The biopsy was taken immediately before and at the end of the bed rest intervention. Following the application of the anaesthetic, a 1.0–1.5 cm incision was made to the skin, subcutaneous tissue and muscle fascia, and the tissue sample was harvested with a Rongeur-Conchotome (GmbH&Co, Zepf Instruments, Dürbheim, Germany). The collected muscle tissue was dissected free of fat and connective tissue and rapidly divided into several portions. A small portion (2.0–6.5 mg wet weight) was used immediately to evaluate mitochondrial respiration \textit{ex vivo} (Pesta & Gnaiger 2012). Measurements were performed in duplicate. The portion of tissue was immediately placed in an ice-cold preservation solution (BIOOPS; Oroboros Instruments, Innsbruck, Austria) (4°C) containing: EGTA-calcium buffer (10 mM) (free Ca\(^{2+}\) concentration 100 nmol L\(^{-1}\)), imidazole (20 mM), taurine (20 mM), K+/4 morpholinoethanesulphonic acid (MES) (20 mM), Mg\(^{2+}\) (2 mM), KCl (130 mM), pH 7.4. The tissue was stored at -80°C until analysis. Mitochondrial respiration was determined using an oxygen electrode (Oroboros Instruments, Innsbruck, Austria) and a Warburg apparatus (Braun Instruments, Utah, USA). ATPase activity was measured in a Beckman Coulter DU-65 spectrophotometer (Fullerton, CA, USA) and a Hitachi U-3010 spectrophotometer (Tokyo, Japan) as described by Pesta & Gnaiger (2012). Mitochondrial respiration was measured in duplicate and the mean values were calculated.

During the recovery from the constant work rate cycling exercise mitochondrial respiration was evaluated \textit{in vivo} by determining the \( \dot{V}\textsubscript{O2}m \) kinetics by NIRS and the repeated occlusions method (Ryan et al. 2012; Adami & Rossiter, 2018; Zuccarelli et al. 2020). The NIRS instrument and the site of application of the NIRS probe were the same described above. The NIRS signals during ischaemia were normalized for changes in blood volume by utilizing the method proposed by Ryan et al. (2012). Before the exercise period a 5 min ischaemic calibration (physiological normalization) was performed by inflating a pressure cuff (∼300 mmHg) positioned at the inguinal crease of the thigh.

\[ y(t) = y_{\text{bas}} + A_u \left[ 1 - e^{-(t-TD_u)/\tau_u} \right] + A_d \left[ 1 - e^{-(t-TD_d)/\tau_d} \right] \]
(50 mM), dithiothreitol (0.5 mM), MgCl2 (6.56 mM), ATP (5.77 mM) and phosphocreatine (15 mM) (pH 7.1). Fibre bundles were trimmed from the connective and fat tissue excess (if present) and separated with sharp-ended needles under magnification (70 ×) (Stereomicroscope CRYSTAL-PRO, Konus-optical & sports systems, Italy). After this, fibre bundles were incubated into 2 mL of BIOPS containing 20 μg mL⁻¹ saponin for 30 min at 4°C with continuous gentle stirring to ensure complete permeabilization. Samples were washed with the respiration medium (MIR05; Oroboros Instruments, Innsbruck, Austria) containing 0.5 mM EGTA, 60 mM potassium lactobionate, 3 mM MgCl₂ 6H₂O, 20 mM taurine, 10 mM KH₂PO₄, 20 mM Hepes, 110 mM sucrose and 1 g L⁻¹ BSA, pH 7.1, weighed in a balance-controlled scale (Shimatzdu). After this, permeabilized fibres were measured for wet weight and immediately transferred into the respirometer (Oxygraph-2k Oroboros Instruments) chambers for O₂ consumption analysis. Mitochondrial respiratory function was evaluated by measuring O₂ consumption polarographically by high-resolution respirometry (Pesta & Gnaiger 2012). Data were digitally recorded using DatLab4 software (Oroboros Instruments). The instrumentation allows for O₂ consumption measurements with small amounts of sample in closed respiration chambers containing 2 mL of air-saturated respiration medium (MIR06; MIR05 + catalase 280 IU mL⁻¹) at 37°C. Standardized instrumental and chemical calibrations were performed to correct for back-diffusion of O₂ into the chamber from the various components (e.g. leak from the exterior, O₂ consumption by the chemical medium and by the sensor O₂) (Pesta & Gnaiger 2012). The O₂ concentration in the chamber was maintained between 280 and 400 μM (average O₂ partial pressure 250 mmHg) to avoid O₂ limitation of respiration. Intermittent reoxygenation steps were performed during the experiments by injections of 1–3 μL of 0.5 mM H₂O₂, which was instantaneously dismutated by catalase, already present in the medium, to O₂ and H₂O. Experiments were performed in the presence of the myosin II-ATPase inhibitor (Blebbistatin, 25 μM, dissolved in DMSO 5 mM stock) (Perry et al. 2011) in order to prevent spontaneous contraction in the respiration medium.

A substrate-uncoupler-inhibitor-titration protocol, with a substrate combination that matches physiological intracellular conditions, was applied (Pesta & Gnaiger 2012; Salvadego et al. 2016, 2018). Non-phosphorylating resting mitochondrial respiration was measured in the presence of malate (4 mM) and glutamate (10 mM) and in the absence of adenylates, so that O₂ consumption was mainly driven by the back leakage of protons through the inner mitochondrial membrane (‘leak’ respiration). Succinate (10 mM) was added to support convergent electron flow into the Q-junction through complexes I and II. This was followed by submaximal titration of ADP (12.5, 25, 175, 250, 500, 1000, 2000, 4000, 6000, 8000, 10,000 μM) to assess complex I+II-linked ADP sensitivity and maximal ADP-stimulated mitochondrial respiration (OXPHOS). Cytochrome C (10 μM) was added to test mitochondrial outer membrane integrity. The addition of cytochrome C had no significant additive effects on respiration, with minor increases of <5%, thereby confirming the integrity of the outer mitochondrial membrane. Maximal uncoupled mitochondrial respiration (electron transport system capacity) was measured by stepwise additions of chemical uncoupler protonophore carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP). Rotenone (1 μM) and antimycin A (2.5 μM) were added to inhibit complexes I and III, providing a measure of residual O₂ consumption, indicative of non-mitochondrial O₂ consumption. Mitochondrial respiration indices were then corrected for O₂ flux resulting from residual O₂ consumption. The degree of coupling of oxidative phosphorylation for a specific substrate supply (glutamate and malate and succinate) was determined by calculating the ratio between OXPHOS and LEAK respiration (Pesta & Gnaiger 2012). The obtained mitochondrial respiration values were also normalized by citrate synthase activity (see below), taken as an estimate index of mitochondrial mass (Larsen et al. 2012).

In order to evaluate the sensibility of mitochondrial respiration to submaximal [ADP] (squared brackets denote concentrations) (Holloway et al. 2018), high-resolution respirometry was used to assess mitochondrial respiration, after giving glutamate, malate and succinate, in the presence of 11 increasing [ADP] (see above). ADP-stimulated respiration values (expressed as a percentage of the maximal values) were analysed by a Michaelis-Menten kinetic equation (eqn (3)):

\[ y = V_{\text{max}} \frac{x}{K_m + x} \]  

(3)

where \( x \) is the [ADP] (μM), \( y \) indicates the mitochondrial respiration (O₂ consumption) at the given [ADP], \( V_{\text{max}} \) is the maximal O₂ consumption and \( K_m \) is the [ADP] providing 50% of the maximal mitochondrial respiration.

Data were also fitted by a biexponential function according to eqn (4):

\[ y(x) = y_{\text{BAS}} + A_1 \left[ 1 - e^{(x-C_{1})/C_{1}} \right] \\
+ A_2 \left[ 1 - e^{(x-C_{2})/C_{2}} \right] \]  

(4)

where \( x \) is [ADP] (μM), \( y_{\text{BAS}} \) indicates the baseline, which was always set to 0, \( A_1 \) is the amplitude between the \( y_{\text{BAS}} \) and the steady state during the first exponential component, \( C_1 \) is the [ADP] at the beginning of the first exponential component, which was always set to 0, and \( C_2 \) is the constant of increase that indicates the [ADP] needed to achieve 63% of the steady state during the
first exponential component. $A_2$, $C_2$ and $CI_2$ indicate the amplitude of the second exponential component, the [ADP] at the start of the second exponential component and the constant of increase of the second exponential component, respectively. A variable equivalent to the apparent $K_m$ (see above) was then calculated by solving eqn (4) in order determine the [ADP] corresponding to 50% of the maximal ADP-stimulated mitochondrial respiration. 

At the conclusion of each experiment, muscle samples were removed from the chamber, immediately frozen in liquid nitrogen and then stored at -80°C until determination of citrate synthase (CS) activity (see below). 

**Citrate synthase activity**

In order to determine CS activity, muscle samples were thawed and underwent a motor-driven homogenization in a pre-cooled 1 mL glass-glass potter (Wheaton, USA). The muscle specimen was suspended 1:50 w/v in a homogenization buffer containing sucrose (250 mM), Tris (20 mM), KCl (40 mM) and EGTA (2 mM) with 1:50 v/v protease (P8340-Sigma) inhibitors. The specimen was homogenized in an ice-bath with 20 strokes at 500 rpm; before the last hit Triton X-100 (0.1% v/v) was added to the solution. After this, the sample was left in ice for 30 min. The homogenate was centrifuged at 14,000 g for 10 min. The supernatant was used to evaluate protein concentration according to the method of Lowry et al. (1951). Protein extracts (5–10–15 μg) were added to each well of a 96-well-microplate along with 100 μl of 200 mM Tris, 20 μl of 1 mM 5, 5'-dithiobis-2-nitrobenzoate (DTNB), freshly prepared, 6 μl of 10 mM acetyl-coenzyme A (Acetyl-Co-A) and mQ water to a final volume of 190 μl. A background ΔAbs, to detect any endogenous activity by acetylace enzymes, was recorded for 90 s with 10 s intervals at 412 nm at 25°C by an EnSpire 2300 Multilabel Reader (PerkinElmer). The ΔAbs was subtracted from the one given after the addition of 10 μl of 10 mM oxalacetic acid that started the reaction. All assays were performed at 25°C in triplicate on homogenates. Activity was expressed as nmol min⁻¹ (mU) per mg of protein. This protocol was modified from (Srere 1969; Spinazzi et al. 2012).

**Femoral artery blood flow during passive leg movements**

Baseline blood flow in the common femoral artery was not affected by bed rest (443 ± 100 mL min⁻¹ in POST vs. 429 ± 96 in PRE; $P = 0.55$). On the contrary, baseline common femoral artery diameter was reduced after bed rest, by about 5% (0.89 ± 0.11 cm in POST vs. 0.94 ± 0.12 in PRE; $P = 0.02$). The PLM results are presented in Fig. 1. Leg blood flow increased immediately after the onset of PLM, reaching a peak both in PRE (1310 ± 467 mL min⁻¹) and in POST (1107 ± 472) after about 10 s. Peak blood flow values in the two conditions were not significantly different ($P = 0.19$). The area under the blood flow vs. time curve (AUC) during PLM was smaller ($P = 0.03$) in POST (274 ± 233 mL) vs. PRE (427 ± 291).

**Statistical analysis**

Data are presented as means ± SD with the exception of Fig. 1 where SEM is used for clarity. Statistical significance of differences between POST and PRE was checked by a two-tailed Student’s $t$ test for paired data. The level of significance was set at $P < 0.05$. Data fittings by exponential functions were performed by the least-squared residuals method. Statistical analyses were carried out with a commercially available software package (Prism 6.0; GraphPad).

**Results**

Each subject completed the entire experimental protocol. Pulmonary $V\text{O}_2$peak was 9% lower in POST (40.3 ± 6.1 ml·kg⁻¹·min⁻¹) vs. PRE (44.5 ± 7.2) ($P = 0.001$). Anthropometric characteristics are reported in Table 1. BM and BMI decreased by ~2% after bed rest. Skin and ATT measured at the site of placement of the NIRS probe ranged between 4.0 and 8.4 mm; the thickness was slightly reduced after bed rest ($P = 0.03$).
Intramuscular matching between O$_2$ delivery and O$_2$ utilization

The upper panels of Fig. 2 show [deoxy(Hb + Mb)] values of a typical subject in PRE and in POST during the first 90 s of very low-intensity exercise, corresponding to about 30 W. An ‘overshoot’ (see Methods) of [deoxy(Hb + Mb)] was evident in the majority of the subjects both in PRE (7 out of 10) and in POST (9 out of 10). The individual and mean (± SD) values of the amplitude of the overshoot are given in the lower panel of Fig. 2. The amplitude was greater ($P = 0.03$) in POST vs. PRE.

Mitochondrial respiration in vivo

Representative repeated arterial occlusion protocols in PRE and POST are shown in Fig. 3. $\dot{V}O_2m$ recovery curves for a typical subject in PRE and in POST are shown in the upper panels of Fig. 4. A monoexponential decrease was observed for all participants. For all experiments the fitting was excellent, and the coefficients of determination ($r^2$) ranged between 0.90 and 0.95. Individual and mean (± SD) values of $\tau$ (time constant) and $k$ (velocity constant) of the $\dot{V}O_2m$ recovery kinetics are shown in the lower panels of Fig. 4. No significant differences of data obtained in POST vs. PRE were observed. In other words, bed rest did not affect the $\dot{V}O_2m$ recovery kinetics (see Fig. 4).

The dashed horizontal lines in the upper panels indicate the $\dot{V}O_2m$ values obtained at rest before the exercise. Resting $\dot{V}O_2m$ values were lower than the asymptotic $\dot{V}O_2m$ values obtained with the fitting; in other words, at the end of the repeated arterial occlusions, $\dot{V}O_2m$ still did not reach the resting values. $\Delta$[deoxy(Hb + Mb)] values during the first occlusion following the exercise were about 30% of the physiological calibration. $\dot{V}O_2m$ values extrapolated at time = 0 (start of the recovery) in the upper panel of Fig. 4 were about 48 and 37 times higher in POST and in PRE, respectively, than resting $\dot{V}O_2m$.

Mean linear regression lines of the $\Delta$[deoxy(Hb + Mb)] increases during the first minute of the blood occlusion manoeuvres performed for the ‘physiological calibration’ (see above) are presented in the upper panel of Fig. 5. Resting $\dot{V}O_2m$ values (see individual and mean (± SD) values in the lower panel of Fig. 5) were about 25% lower in POST (0.057 ± 0.02 μM L$^{-1}$ s$^{-1}$) vs. PRE (0.078 ± 0.02) ($P = 0.006$).

Mitochondrial respiration ex vivo

Individual and mean (± SD) data of CS activity are shown in Fig. 6. No significant differences were observed in POST vs. PRE. Being aware of the potential problem of determining CS activity in a very small muscle bundle (max 6.5 mg wet weight) which for ~1–1.5 h
was kept at 37°C for the respiratory measurements, we performed pilot experiments (unpublished observations) in which we compared CS measurements determined on samples taken from the same muscle but undergoing two different procedures: in one case the CS measurements were performed (as in the present study) on material frozen after it was taken from the chamber following the respirometry experiments, whereas in the ‘control’ condition, the CS measurements were performed on samples immediately frozen after the biopsy. The results did not show significant differences between the two measurements: 410 ± 50 nmol min⁻¹ mg protein vs. 390 ± 50, n = 10, P = 0.23.

The main data related to mitochondrial respiration (JO₂) ex vivo obtained by high-resolution respirometry are presented in Fig. 6. The data were expressed per mg of wet weight. Although there were inter-individual variations in the responses to bed rest, for all variables there were no significant changes in POST vs. PRE. Mitochondrial leak respiration (‘LEAK’), maximal ADP-stimulated mitochondrial respiration (‘OXPHOS’), supported by complex I and complex II, and the maximal uncoupled mitochondrial respiration supported by complex I and complex II (‘ETS’) were not significantly different in POST vs. PRE. Also rotenone-insensitive ETS, the mitochondrial electron transport system capacity supported by complex II, was not significantly different (P = 0.09) in PRE (46.6 ± 13.2 pmol s⁻¹ mg⁻¹) vs. POST (38.6 ± 10.4).

The general message (no significant differences in mitochondrial respiration variables in POST vs. PRE) was confirmed after LEAK, OXPHOS and ETS respiration values were normalized per unit of CS activity. LEAK/CS was 0.149 ± 0.054 pmol s⁻¹ mU⁻¹ in POST and 0.140 ± 0.055 in PRE (P = 0.67); OXPHOS/CS was 0.509 ± 0.134 pmol s⁻¹ mU⁻¹ in POST and 0.534 ± 0.135 in PRE (P = 0.61) and ETS/CS was 0.485 ± 0.161 pmol s⁻¹ mU⁻¹ in POST and 0.579 ± 0.158 in PRE (P = 0.14).

Also, the mitochondrial coupling of oxidative phosphorylation (‘respiratory control ratio’), calculated by the ratio OXPHOS/LEAK was not significantly different in POST vs. PRE (see Fig. 6; P = 0.44). The ratio between OXPHOS and ETS (‘respiratory system control ratio’) was significantly higher in POST compared with PRE (see Fig. 6). In PRE, ETS capacity was slightly but significantly

![Figure 3. Repeated arterial occlusion protocol](image)

**Figure 3. Repeated arterial occlusion protocol**

Typical examples of the changes in [deoxy(Hb+Mb)] values during the first 12 ischaemia before (PRE, upper panel) and after bed rest (POST, lower panel). Dashed lines represent the recovery of muscle VO₂ over time.

![Figure 4. Assessment of in vivo mitochondrial function](image)

**Figure 4. Assessment of in vivo mitochondrial function**

In the upper panel, muscle VO₂ (VO₂m) data during the recovery from moderate-intensity exercise are shown for a representative subject before (PRE) and after (POST) bed rest. The inset shows mitochondrial respiration in response to the first ADP titrations. The fitted functions and the calculated time constant (τ) values (see Methods) are also shown. The dotted horizontal lines indicate the resting baseline values. In the lower panel, individual and mean (± SD) values of the time constant and rate constant (k) of muscle VO₂ (VO₂m) recovery kinetics before (PRE) and after (POST) bed rest are shown. No statistically significant differences were observed. See text for further details. [Colour figure can be viewed at wileyonlinelibrary.com]
higher than OXPHOS (+8%; \( P = 0.01 \)), indicating that the phosphorylating system exerted a control over coupled respiration (Pesta & Gnaiger 2012). This control disappeared after bed rest (+0% between ETS capacity and OXPHOS; \( P = 0.36 \)) (see Fig. 6).

High-resolution respirometry was also used to assess the sensitivity of mitochondrial respiration to submaximal [ADP] (Holloway et al. 2018). Data were first analysed according to Michaelis–Menten kinetics (see Methods), as previously described (see, e.g. Perry et al. 2011; Holloway et al. 2018). The upper panel of Fig. 7 shows a typical example of the ADP-stimulated respiration data fitted by this approach. Although the coefficient of determination \( (r^2) \) was relatively high (0.83), the fitting was obviously not satisfying. Analysis of residuals (see inset panels) shows that the fitting markedly overestimates JO2 data from 1000 to 2000 \( \mu \)M, and markedly underestimates JO2 data from 6000 to 10,000 \( \mu \)M. The same typical example was fitted with a double exponential model (see eqn (4) and lower panel of Fig. 7) and the quality of the fitting markedly improved, as also confirmed by the analysis of residuals (see also the Discussion).

We therefore opted for the second fitting approach, and the fittings performed on the mean JO2 values are shown for PRE and POST in Fig. 8. The high quality of the fitting was confirmed. A greater sensitivity of JO2 to submaximal [ADP] in POST vs. PRE is evident from the figure: for the same submaximal [ADP], higher JO2 values are present in POST. Equation (3) was then applied to the individual values, and the equations were solved in order to calculate the [ADP] values corresponding to 50% of maximal JO2. This parameter should have the same meaning of the \( K_m \) obtained with the classic Michaelis–Menten equation. [ADP] at 50% of JO2max was significantly lower in POST vs. PRE (see individual and mean (± SD) values in the lower panel of Fig. 8), confirming the increased sensitivity of JO2 to submaximal [ADP] in POST vs. PRE.

**Discussion**

The general aim of the present study was to identify peripheral biomarkers of impairment of oxidative metabolism during exercise following a relatively short (10 days) exposure to horizontal bed rest, with specific attention toward the respective roles of microvascular–endothelial function and mitochondrial respiration. The main sites of impairment were located upstream of mitochondria, at the level of microvascular and endothelial functions and in the intramuscular matching between \( O_2 \) delivery and \( O_2 \) uptake, whereas mitochondrial mass estimated only ex vivo and respiratory activity, both in vivo and ex vivo, were substantially unaffected. For some aspects (mitochondrial sensitivity to submaximal [ADP]) mitochondrial function was even enhanced by bed rest. An unexpected and interesting observation was represented by the decreased resting \( V_{O_2}m \) after bed rest.

Microvascular–endothelial function was evaluated by the method recently proposed by Gifford and Richardson (2017). The blood flow increase (determined by Doppler ultrasound) in the common femoral artery during a 1 min period of passive knee flexion–extension (PLM) has been determined in young untrained and trained subjects, untrained and trained older adults, patients with chronic heart failure (Gifford & Richardson, 2017) and patients with chronic obstructive pulmonary disease (Ives et al. 2020). The blood flow increase during PLM was higher in trained vs. untrained subjects, higher in young vs. old subjects, lower in patients vs. healthy controls. Moreover, the blood flow increase during PLM was well correlated with indices of nitric oxide availability and endothelial function (Gifford & Richardson, 2017), a critical index of general cardiovascular health. According to Gifford & Richardson (2017), although the PLM-induced response is measured in the common femoral artery, its strong relationship with
acetylcholine-induced dilatation of the microvasculature, a common marker of microvascular function, points to a more distal site of functional evaluation compared with flow-mediated vasodilation (FMD), classically considered a biomarker of conduit artery function.

In our study we observed a less pronounced blood flow increase during PLM after 10 days of bed rest (see Fig. 1), suggesting an early impairment of microvascular/endothelial function following simulated microgravity–inactivity. Interestingly, the blood flow increase during PLM observed in our young subjects after 10 days of bed rest was not substantially different from that described by Gifford and Richardson (2017) in subjects of 60–70 years of age. Moreover, the less pronounced increase in blood flow during PLM after bed rest was associated with a reduction of the common femoral artery lumen diameter. In the present study, 10 days of bed rest led to a 5% decrease (0.5 mm) in resting lumen diameter of the vessel. A very similar decrease (6%) was detected after 7 days of leg casting (Sugawara et al. 2004). This phenomenon has also been reported across a spectrum of different modalities of physical inactivity, ranging from spinal cord injury patients, bed rest and unilateral lower limb suspension (Thijssen et al. 2011). Changes in femoral artery diameter are also associated with increases in wall thickness, with the result of promoting atherosclerotic progression (Thijssen et al. 2011). A decreased leg blood flow was described before following a short period of bed rest (Mikines et al. 1991).

In the present study, microvascular function was evaluated by also utilizing another biomarker, related to the intramuscular matching between O₂ delivery and O₂ uptake. A critical issue of peripheral oxidative metabolism, particularly during metabolic transitions (rest-to-exercise, lower-to-higher intensity exercise), deals with the temporal and spatial intramuscular matching between O₂ delivery and O₂ uptake (Grassi et al. 2019), in which nitric oxide plays a critical role (Poole et al. 2012). A ‘weak spot’ in this matching resides in the very different morphological and functional organization of muscle fibre recruitment, based upon the motor unit, and of microvascular recruitment, based upon the ‘microvascular unit’ (Segal 1999). During metabolic transitions a suboptimal intramuscular matching between O₂ delivery and O₂ uptake could lead to a transient underperfusion of areas of muscles, and possibly to a transient overperfusion of other regions. The resulting O₂ delivery-to-O₂ uptake mismatch could determine a transiently increased (‘overshoot’) microvascular fractional O₂ extraction, which has been repeatedly demonstrated by NIRS in pathological conditions (Porcelli et al. 2014, 2016; Grassi et al. 2019), as well as in subjects exposed to bed rest periods of longer duration (Porcelli et al. 2010, Salvadego et al. 2018). The overshoot of fractional O₂ extraction would represent a ‘mirror image’ of the undershoot of microvascular O₂ partial pressure (PO₂) described by phosphorescence quenching (Poole et al. 2012). A lowering of microvascular PO₂, albeit transitory, would decrease O₂ driving pressure for blood to myocyte O₂ flux, thereby impairing peripheral O₂ diffusion and skeletal muscle oxidative metabolism.

In the present study the intramuscular matching between O₂ delivery and O₂ uptake was evaluated by analyzing the dynamic profiles of [deoxy(Hb + Mb)] during the first 60 s of low-intensity constant work rate cycling exercise, as previously described (Porcelli et al. 2010, 2014, 2016; Salvadego et al. 2018). After 10 days of horizontal bed rest, the time-course of [deoxy(Hb + Mb)] was characterized by a greater transient overshoot.

Figure 6. Assessments of ex vivo mitochondrial function and mass
Individual and mean (± SD) values of mitochondrial respiration variables obtained by high-resolution respirometry in permeabilized skeletal muscle fibres are shown; data were obtained before (PRE) and after (POST) bed rest. In the upper panel: LEAK respiration, OXPHOS capacity, and electron transport system (ETS) capacity. In the lower panel: respiratory control ratio (OXPHOS/LEAK) and citrate synthase (CS) activity. No statistically significant differences were observed. See text for further details. [Colour figure can be viewed at wileyonlinelibrary.com]
compared with measurements before bed rest, implying an impaired matching between intramuscular O₂ delivery and metabolic demand. This microvascular impairment therefore appears to be in agreement with that identified by the PLM approach described above, and definitively points to a microvascular impairment following 10 days of bed rest.

Mitochondrial function evaluated by high-resolution respirometry on isolated and permeabilized non-contracting skeletal muscle fibres (Perry et al. 2011; Pesta & Gnaiger 2012), showed similar results to those observed by Salvadego et al. (2016): no variables were affected by bed rest (see Fig. 6). ‘Leak’ respiration, which represents the dissipation of the H⁺ gradient across the inner mitochondrial membrane, not associated with phosphorylation of ADP, was not different following bed rest. Maximal ADP-stimulated mitochondrial respiration (OXPHOS) supported by complex I and complex II, determined in the presence of saturating ADP levels and unlimited substrates and O₂ availability, was unchanged as well. Also, the maximal capacity of electron transport system (ETS), uncoupled from the phosphorylating system, the rotenone-insensitive ETS and oxidative phosphorylation coupling were not affected. Interestingly, the uncoupled mitochondrial respiration was significantly higher than oxidative phosphorylation capacity only before bed rest (see Fig. 6). An increased oxygen flux following the uncoupling of mitochondrial respiration is expected to occur in skeletal muscle fibres when the phosphorylation system (i.e. adenine nucleotide translocase, ANT, phosphate carrier and ATP synthase) limits OXPHOS capacity (Pesta & Gnaiger 2012). In the present study it seems that following bed rest the capacity of the phosphorylation pathway fully matches ETS capacity, such that OXPHOS capacity was not limited by the phosphorylation system or by the proton backpressure. This phenomenon typically occurs in mouse skeletal and cardiac tissue (Gnaiger et al. 2009; Lemieux et al. 2017). Further investigations are needed to clarify the mechanisms responsible for these observations.

The absence of significant differences was also confirmed after the respirometric data were normalized per unit of CS activity, taken as an index of mitochondrial mass (Larsen et al. 2012), which was not affected by bed rest either. In previous studies, the effects of short periods of bed rest on maximal ADP-stimulated mitochondrial respiration are somehow controversial. Whereas Miotto et al. (2019) and Dirks et al. (2020) described an impaired mitochondrial function following bed rest periods of 3 and 7 days, respectively, other authors (Salvadego et al. 2016; Larsen et al. 2018) did not see impairments following 4 and 10 days of bed rest. No hypotheses can be forwarded to explain the conflicting data. It should be mentioned that in the present study the absence of differences of mitochondrial respiration following bed rest was also confirmed by the biomarker obtained in vivo (see below). According to data obtained by a different group belonging to the present project (Sandri et al., unpublished observations) a substantial impairment of the ‘transcriptome’ of mitochondrial genes occurs as early as after 5 days of bed rest. The contradiction with our results, however, could be only apparent, in the sense that changes at the level of the transcriptome may become evident before functional changes are observed. The scenario could be different with a more prolonged exposure to simulated microgravity, a condition in which both respirometric (Salvadego et al. 2018) and proteomic data (Brocca et al. 2012; Buso et al. 2019) are in favour of altered mitochondrial function and structure.

In the present study we also investigated the sensibility of mitochondrial respiration to submaximal (and physiological) [ADP]. Free [ADP] in skeletal muscle ranges between 25 and 250 μM (Howlett et al. 1998), way below the unlimiting [ADP] utilized to evaluate maximal ADP-stimulated mitochondrial respiration (OXPHOS). In the present study we utilized increasing [ADP], starting from 12.5 μM, thereby including also

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'physiological' [ADP] values. The parameter equivalent to the apparent $K_m$ ([ADP] at 50% of $JO_2\text{max}$) was estimated by solving a biexponential function, which provided a better fit of experimental data compared with the traditional fitting for $K_m$ estimation according to Michaelis–Menten kinetics (see Fig. 7). Analysis of previous studies which applied the Michaelis–Menten kinetics model (see, e.g. Perry et al. 2011; Holloway et al. 2018) to fit $JO_2$ vs. [ADP] data confirms the unsatisfactory fitting. At the moment we do not know if the new fitting we are proposing represents only a mathematical improvement, or if it can give insights into cellular mechanisms regulating mitochondrial respiration, possibly related to the presence of different fibres with different respiratory sensitivity to [ADP], and/or to the presence of multiple regulatory factors. The measured $JO_2$ in the presence of ADP represents indeed the final product of complex reactions, and not a single reaction. These complex reactions involve at least four components: transport of nucleotides across external and internal mitochondrial membrane, transport of phosphate, ATP synthase activity and respiratory chain activity (Mitchell & Moyle, 1967; Guzun et al. 2012; Letts & Sazaov, 2017).

In any case, in the present study, whereas maximal ADP-stimulated mitochondrial respiration and other respirometric variables remained unchanged after bed rest, sensitivity of mitochondrial respiration to sub-maximal [ADP] was enhanced, as shown by the leftward shift of the $JO_2$ vs. [ADP] relationship (higher $JO_2$ for the same [ADP] after bed rest) and by the lower values of [ADP] at a $JO_2$ corresponding to 50% of max. A conclusive interpretation of these data is still lacking, but they are in accordance with literature findings, showing a decrease in ADP sensitivity (higher $K_m$) of skeletal muscle fibres following training (Walsh et al. 2001). The present data also confirm the results obtained by Dirks et al. (2020) following a bed rest period of similar duration. In that study the observation was associated with a reduction in OXPHOS protein content, but with an unchanged content of proteins involved in the transport of ADP across the outer (voltage-dependent anion channel, VDAC) and inner (adenine nucleotide translocase, ANT) mitochondrial membranes (Dirks et al. 2020). Considering that we observed no reduction in either mitochondrial mass (CS activity) or state III respiration, no reduction in protein content should be involved of either OXPHOS and mitochondrial proteins involved in ADP transport across the outer (i.e. VDAC) and inner (i.e. ANT) membranes. Conversely, we can postulate that post-translational modifications/functional regulation of mitochondrial membrane transporters are the more likely to be responsible for the effect. Further investigations are needed to verify such a tempting hypothesis. The reduced apparent $K_m$ after bed rest could be interpreted as an attempt to maintain metabolic homeostasis (Dirks et al. 2020) in the presence of impairments upstream of mitochondria, as discussed above.

In the present study, mitochondrial mass, as estimated by CS activity, was not affected by bed rest. Also in quantitative terms, therefore, mitochondria were not

![Figure 8. Assessments of mitochondrial ADP sensitivity](image-url)
affected. This observation confirms previous observations by our group and others following a relatively short bed rest period (Salvadego et al. 2016; Dirks et al. 2020), but it is in disagreement with other observations in which CS activity was reduced after 4 and 7 days of bed rest (Dirks et al. 2016; Larsen et al. 2018). With prolonged periods of bed rest, mitochondrial mass was reduced after 4 weeks and 42 days of bed rest (Berg et al. 1993; Ferretti et al. 1997).

The absence of impairments of mitochondrial respiration, observed ex vivo by high-resolution respirometry on isolated skeletal muscle fibres, was also confirmed in vivo, non-invasively, by the analysis of the kinetics of $\dot{V}O_2m$ recovery following cycle ergometer exercise, carried out by NIRS and the repeated occlusions method (Ryan et al. 2012; Adami & Rossiter, 2018; Zuccarelli et al. 2020). $\dot{V}O_2m$ recovery kinetics is a mirror image of phosphocreatine recovery kinetics (Ryan et al. 2013), which is considered a classic evaluation tool for intramuscular oxidative metabolism (Kent & Fitzgerald, 2016). Methodological improvements in the method adopted in the present study, with respect to the conventional approach (Ryan et al. 2012, Adami & Rossiter, 2018), are discussed in detail in a previous study by our group (Zuccarelli et al. 2020). To the best of our knowledge, this is the first time that this methodology has been applied after bed rest. The time constant ($\tau$) of $\dot{V}O_2m$ recovery kinetics was not affected by bed rest (see Fig. 4), thus confirming that skeletal muscle mitochondrial function was not compromised.

An unexpected and interesting finding of the present study is related to the resting $\dot{V}O_2m$, measured non-invasively by calculating the linear slope of muscle deoxygenation (determined by NIRS) during a transitory limb ischaemia induced by the rapid inflation of a pneumatic cuff. A significant decrease (of about 25%) in resting $\dot{V}O_2m$ was observed following bed rest. A decrease in whole body resting $\dot{V}O_2$ of about 7% after 7 weeks of immobilization has been reported by Deitrick et al. (1948), and it was subsequently confirmed by other studies (Teasell & Dittmer, 1993; Downs et al. 2020). No similar data are available for immobilization periods of shorter duration. Disuse/immobilization is known to decrease muscle protein synthesis and increase muscle protein degradation, leading to skeletal muscle atrophy (Crossland et al. 2019; Degens et al. 2019). Data obtained by other groups of the present research project indicate a decrease in quadriceps femoris volume by about 5% (Monti et al. 2021). As far as we know, the present data are the first to demonstrate a decreased resting $\dot{V}O_2m$ induced by bed rest. Anabolic reactions in muscle are energetically more expensive than catabolic ones. Thus, a reduction of muscle protein synthesis, even in the presence of an enhanced protein degradation, would decrease the energy expenditure in resting muscle. It is noteworthy that the decreased resting energy expenditure in skeletal muscle was quantitatively significant (-25%) after only 10 days of bed rest. It would be of interest to determine this variable after longer bed rest periods. It is intriguing to note, in this respect, that a state of ‘metabolic depression’ (about a 20% decrease of metabolic rate relative to basal levels) has been recently proposed as a condition mitigating the biological and logistical challenges of human spaceflight (Regan et al. 2020).

The reduction in resting $\dot{V}O_2m$ observed in the present study could also be the result of a higher muscle oxidative phosphorylation efficiency at rest (i.e. lower $O_2$ cost of ATP resynthesis). In this regard, however, no changes in leak mitochondrial respiration or in the respiratory control ratio were detected in the present study, suggesting that immobilization plays a role in decreasing energy expenditure at rest, rather than increasing muscle mitochondrial efficiency.

The decreased resting $\dot{V}O_2m$ observed in the present study after bed rest cannot be attributed to muscle atrophy. The volume of the small and superficial area of muscle investigated by the NIRS probe (Grassi & Quaresima, 2016) is indeed the same, irrespective of muscle atrophy. If anything, the small decrease in ATT observed after bed rest would increase the relative proportion of muscle vs. adipose tissue investigated by the probe. Since energy turnover in skeletal muscle tissue is greater than that in adipose tissue, this would go in the direction of underestimating the observed resting $\dot{V}O_2m$ decrease after bed rest.

Some limitations are present in the study: (i) no analysis of relevant protein involved in both oxidative metabolism and angiogenic processes and capillarization have been performed; (ii) respirometric measurements were not extended to mitochondrial-derived reactive oxygen species production; and (iii) resting muscle $\dot{V}O_2$ measurements, estimated by NIRS during transient ischaemic periods, were not corroborated by other independent measurements, such as resting energy expenditure.

In conclusion, the main peripheral limitations to oxidative metabolism after a 10-day horizontal bed rest were ‘upstream’ of mitochondrial function, at the level of microvascular $O_2$ delivery. Substantial impairments were observed for biomarkers of microvascular/endothelial function, and for the intramuscular matching between $O_2$ delivery and $O_2$ uptake. On the other hand, mitochondrial content and mitochondrial respiration were unaffected or even improved (i.e. enhanced mitochondrial sensitivity to submaximal [ADP]) following bed rest. An unchanged mitochondrial respiration was also confirmed by a biomarker obtained in vivo ($\dot{V}O_2m$ recovery kinetics). The decreased resting $\dot{V}O_2m$ (by about 25%) following bed rest could represent an adaptive phenomenon in response to simulated microgravity–inactivity,
attributable to the fact that muscle catabolic processes within muscles are less expensive, in terms of energy, than anabolic ones. The concepts mentioned above, besides being of interest from a basic science point of view, may be relevant for pathological conditions characterized by relatively short periods of profound inactivity, and they could affect the definition of countermeasures or rehabilitative interventions. Future studies should be conducted in order to determine the potential causes of the interindividual variation in some physiological responses to bed rest. These studies could at least in part explain why some astronauts experience greater functional impairments in space or upon returning to Earth, and could also be useful in the selection of the astronauts.

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Additional information

Data availability statement

All relevant data are presented as individual data points in the figures. Data not presented with individual data points are available from the corresponding author upon request.

Competing interests

No competing interest are declared.

Author contributions

B.G. and M.N. conceived the study and obtained the financial support. B.S. and R.P. were responsible for the organization of the bed rest campaign. B.G., L.Z. and L.R. contributed to the experimental design. L.Z., G.B., B.M., C.D., M.G., G.M., M.M., A.P., S.P. and L.R. collected the data. L.Z., G.B., B.M., C.D., G.M. and B.G. analysed the data. M.C. and I.M. contributed to the *ex vivo* mitochondrial respiration analysis. L.Z. and B.G. wrote the original draft. All authors critically revised the draft and approved the final version of the manuscript.

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Keywords

bed rest, microgravity, mitochondrial respiration, NIRS, oxidative metabolism, PLM, skeletal muscle

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

Peer Review History

Statistical Summary Document