Respiratory Physiology & Neurobiology 172 (2010) 53–62

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Cardiovascular determinants of maximal oxygen consumption in upright and supine posture at the end of prolonged bed rest in humans

Aurélien Bringard[a], Silvia Pogliaghi[b], Alessandra Adami[a,b], Gabriela De Roia[b], Frédéric Lador[c], Daniela Lucini[d], Paolo Pizzinelli[d], Carlo Capelli[b], Guido Ferretti[a,e,*]

[a] Département des Neurosciences Fondamentales, Centre Médical Universitaire, 1 rue Michel Servet, CH-1211 Geneve 4, Switzerland
[b] Facoltà di Scienze dell'Esercizio e dello Sport, Università di Verona, Via Felice Casorati 43, I-37131 Verona, Italy
[c] Division de Pneumologie, Département de Médecine Interne, Hôpitaux Universitaires de Genève, 24 rue Micheli-du-Crest, CH-1211 Geneve 14, Switzerland
[d] Centro Terapia Neurovegetativa, Dipartimento di Scienze Cliniche, Ospedale L. Sacco, Università di Milano, Via G.B. Grassi 74, I-20157 Milano, Italy
[e] Department of Biomedical Science and Biotechnology, University of Brescia, Viale Europa 11, I-25123 Brescia, Italy

ARTICLE INFO

Article history:
Accepted 19 March 2010

Keywords:
Exercise capacity
Cardiac output
Cardiovascular response
Simulated microgravity

ABSTRACT

We tested the hypothesis that, after bed rest, maximal oxygen consumption ($\dot{V}_{O_2max}$) decreases more upright than supine, because of adequate cardiovascular response supine, but not upright. On 9 subjects, we determined $\dot{V}_{O_2max}$ and maximal cardiac output ($\dot{Q}$) upright and supine, before and after (reambulation day upright, the following day supine) 35-day bed rest, by classical steady state protocol. Oxygen consumption, heart rate (f_H) and stroke volume (Q_{st}) were measured by a metabolic cart, electrocardiography and Modelflow from pulse pressure profiles, respectively. We computed $\dot{Q}$ as f_H times Q_{st}, and systemic oxygen flow ($\dot{Q}a_{O_2}$) as $\dot{Q}$ times arterial oxygen concentration, obtained after haemoglobin and arterial oxygen saturation measurements. Before bed rest, all parameters at maximal exercise were similar upright and supine. After bed rest, $\dot{V}_{O_2max}$ was lower ($p < 0.05$) than before, both upright (−38.6%) and supine (−17.0%), being 30.8% higher supine than upright. Maximal Q_{st} decreased upright (−44.3%), but not supine (+3.7%), being 98.9% higher supine than upright. Maximal $\dot{Q}$ decreased upright (−45.1%), but not supine (+9.0%), being higher supine than upright (+98.4%). Maximal $\dot{Q}a_{O_2}$ decreased upright (−37.8%), but not supine (+14.8%), being higher (+74.8%) upright than supine. After bed rest, the cardiovascular response (i) did not affect $\dot{V}_{O_2max}$ supine, (ii) partially explained the $\dot{V}_{O_2max}$ decrease upright, and (iii) caused the $\dot{V}_{O_2max}$ differences between postures. We speculate that impaired peripheral oxygen transfer and/or utilisation may explain the $\dot{V}_{O_2max}$ decrease supine and the fraction of $\dot{V}_{O_2max}$ decrease upright unexplained by cardiovascular responses.

1. Introduction

When a subject stands up again after prolonged bed rest, an event that mimics the return onto Earth of astronauts after space flight, a larger amount of blood than before bed rest is displaced into the blood vessels of his lower limbs, when moving from supine to upright, because he has more compliant lower limb blood vessels (Belin de Chantemèle et al., 2004). Thus, the stroke volume of the heart (Q_{st}) is strongly decreased, and its reduction is further enhanced by the remarkable reduction of blood volume (Johansen et al., 1997). Tachycardia is unable to compensate for this decrease, also because of reduced baroreflex sensitivity (Convertino et al., 1990; Linnarsson et al., 2006; Ferretti et al., 2009). As a consequence, cardiac output ($\dot{Q}$) goes down, both at rest and at submaximal exercise (Ferretti et al., 1998; Spaak et al., 2005; Capelli et al., 2008). When maximal exercise is performed, the coupling of maximal heart rate (f_H) – an invariant – with reduced maximal Q_{st} implies a drastic decrease in maximal $\dot{Q}$ and maximal systemic oxygen delivery ($\dot{Q}a_{O_2}$) (Ferretti et al., 1997). As a consequence, the maximal oxygen consumption ($\dot{V}_{O_2max}$) upright is lower than before bed rest (Saltin et al., 1968; Stremel et al., 1976; Friman, 1979; Convertino et al., 1982; Kashihara et al., 1994; Ferretti et al., 1997; Mekjavic et al., 2005; Capelli et al., 2006; Lee et al., 2007; Ferretti and Capelli, 2009). The decrease in $\dot{V}_{O_2max}$ is visible already after three days in bed only (Smorawinski et al., 2001) and is larger the longer the bed rest duration (Capelli et al., 2006). A similar $\dot{V}_{O_2max}$ reduction in upright posture occurs also after space flight (Levine et al., 1996), despite the countermeasures normally applied in-flight, and represents a major problem for astronauts upon return to Earth, because it entails a remarkable loss of exercise capacity. Yet the

* Corresponding author at: Département des Neurosciences Fondamentales, Centre Médical Universitaire, 1 rue Michel Servet, CH-1211 Geneve 4, Switzerland.
Tel.: +41 22 3795363; fax: +41 22 3795402.
E-mail address: Guido.Ferretti@unige.ch (G. Ferretti).

1569-9048/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.resp.2010.03.018

$\dot{V}_{O_2\,max}$ decrease in upright posture after bed rest might even be larger than reported in the studies cited above, were $\dot{V}_{O_2\,max}$ was measured shortly after standing up at the end of bed rest (day of re-ambulation, $R+0$). In fact in most of those studies $\dot{V}_{O_2\,max}$ was determined at least 3 days ($R+3$) after standing up, whereas the recovery of cardiovascular function is a fairly rapid phenomenon (Spaak et al., 2005).

If what precedes describes the scenario occurring in upright posture, where the hydrostatic force moves blood toward the feet, then a remarkably different picture should be expected after bed rest in the *supine* posture. In fact large venous return, with elevated Q_{st} and bradycardia, occurs as soon as the supine posture is resumed, maintaining submaximal $\dot{Q}$ at the same level as before bed rest (Sundblad et al., 2000; Spaak et al., 2005; Linnarsson et al., 2006), at least up to 60 days of bed rest. Only after 120 days in bed a slight decrease in $\dot{Q}$ was observed (Spaak et al., 2005). For the same maximal f_H, this would lead to the hypothesis of unchanged supine maximal $\dot{Q}$ after bed rest, so that $\dot{V}_{O_2\,max}$ would decrease much less, if not at all, when it is measured supine rather than upright after bed rest. Indeed the few investigations of $\dot{V}_{O_2\,max}$ supine instead of upright during and after bed rest of varying duration (Greenleaf et al., 1989; Trappe et al., 2006) showed a significant decrease in $\dot{V}_{O_2\,max}$, in apparent contrast with this hypothesis. However, optimal testing of the above hypothesis requires a determination of maximal $\dot{Q}$ and $\dot{V}_{O_2\,max}$ in both postures, on the same subjects before and after a given bed rest campaign, in order to analyze the effects of posture on $\dot{V}_{O_2\,max}$ after cardiovascular adaptation to microgravity. To the best of our knowledge, no study of this type has been performed so far. Thus, we carried out the present investigation, the aim of which was to measure maximal $\dot{Q}$, $\dot{V}_{O_2\,max}$ and their cardiovascular determinants in both the supine and the upright posture, before and at the end of prolonged head-down tilt bed rest without countermeasures. Our expectations were that $\dot{V}_{O_2\,max}$ would decrease much less supine than upright after bed rest, and that the differences in $\dot{V}_{O_2\,max}$ between the two postures would entirely be due to differences in maximal $\dot{Q}$ and $\dot{Q}a_{O_2}$.

2. Methods

2.1. Subjects

At start, a total of 10 healthy young male subjects were admitted to the study. After bed rest, one subject had an ankle distortion by falling from a force platform during a stance trial carried out by the medical service just before the test, so that he could not perform maximal exercise tests and had to be excluded from the study. The nine who completed the protocols were 23.1 (±2.5) years old and 179.0 (±7.0) cm tall. Their weight was 75.5 (±10.3) and 72.2 (±9.2) kg, respectively, before and after bed rest. None of them underwent physical or pharmacological countermeasures during the bed rest period, nor were they taking cardiovascular medication at the time of the study. All the subjects were non-smokers and underwent a cardiopulmonary stress test during the selection process. Study designs and methods were approved by the local ethical committee. All the subjects were informed about the aims of the investigation and the methods applied in the experiments, and they all signed a written informed consent form.

2.2. Study design

The bed rest campaign was organized by the Italian Space Agency and by the University of Primorska, Kopar, Slovenia, and took place in summer 2008 at Ankaran, Kopar, Slovenia, in controlled hospital environment. Bed rest was head-down tilt with an incline of −6° and lasted 35 days. No countermeasures were applied during the bed rest period. Measurements were carried out before bed rest and at the end of bed rest. In the latter case, the tests upright were carried out on day $R+0$, one hour after the subjects stood up again. The tests supine were carried out the following day ($R+1$), at the same time of the day as at $R+0$ upright. Random partition of upright and supine tests between $R+0$ and $R+1$ was not allowed due to potential cross effects related to fatigue on other studies integrated in the same bed rest program.

2.3. Protocol

Both upright and supine, $\dot{V}_{O_2\,max}$ was determined during graded intermittent exercise on an electrically braked cycle ergometer (Excalibur, Lode, Groningen, The Nederland), adapted for use in supine posture as well. When used supine, the centre of rotation of the crank axis was located about 15 cm above the level of the heart. A shortened version of the classical intermittent protocol with steady state exercise of increasing intensity (Åstrand et al., 2003) was applied. Similar protocols were used in previous bed rest studies (Saltin et al., 1968; Ferretti et al., 1997; Capelli et al., 2006). Three submaximal work loads were performed, each lasting 6 min. The power levels were 50, 100 and 150 W. This last, however, appeared to be supramaximal in some subjects after bed rest in upright posture and was skipped on them. Then, after having estimated the maximal expected power from the relationship between steady state heart rate and power, a power equivalent to 105% of the estimated maximum was applied, and the subject was asked to maintain it as long as he could (mean duration 296 ± 13 s and 252 ± 69 s after bed rest upright and supine, respectively). Oxygen consumption ($\dot{V}_{O_2}$) was determined continuously on a breath-by-breath basis. Arterial blood pressure and heart rate were continuously recorded on a beat-by-beat basis. The resting steady state values were determined as the mean of the 2 min at rest that preceded the first work load. The exercise steady state values were obtained as the mean during the sixth minute of each work load. At the highest work load, the mean values during the last minute of exercise were retained. Successive work loads were separated by 5 min recovery intervals, during which blood samples were taken at minutes 1, 3 and 5 for the determination of blood lactate concentration. Before bed rest, these recovery periods were at rest. After bed rest, resting recovery could be done only in supine tests. For upright tests, because all the subjects suffered of orthostatic intolerance, active recovery had to be allowed, in order to prevent them from fainting, so during recovery free-wheel pedalling was performed instead of resting.

Individual $\dot{V}_{O_2\,max}$ was established from the plateau attained by the relationship between $\dot{V}_{O_2}$ and power. This plateau was observed in 67% of cases. The remainder 33% included also those tests after bed rest in upright posture in which the protocol had to be completed within three work loads. When the plateau was not observed, the highest observed $\dot{V}_{O_2}$ value was retained as the subject's $\dot{V}_{O_2\,max}$ when at least two out of the following conditions were observed: (1) a lack of increase in f_H between successive work loads; (2) gas exchange ratio values above 1.1; (3) lactate values higher than 10 mM (except after bed rest in upright posture, due to active recovery). The maximal aerobic power ($\dot{w}_{max}$) was then calculated as the lowest power requiring a $\dot{V}_{O_2}$ equal to $\dot{V}_{O_2\,max}$.

2.4. Methods and techniques

$\dot{V}_{O_2}$, carbon dioxide output ($\dot{V}_{CO_2}$), gas exchange ratio (R) and expired ventilation ($\dot{V}_E$) were determined from respiratory gas fractions and ventilatory flow recorded at the mouth using a metabolic cart (Quark b2, Cosmed, Rome, Italy). The metabolic system was calibrated before and after each experimental session by means of

Table 1
The metabolic and respiratory data obtained at maximal exercise before and after the bed rest in upright and supine posture.

	Upright		Supine	
	PRE	POST	PRE	POST
$\dot{V}_{O_{2max}}$ (l min^{-1})	3.14 ± 0.40	1.93 ± 0.20*	3.04 ± 0.41	2.52 ± 0.26*,$
$\dot{w}_{max}$ (W)	251 ± 37	142 ± 22*	223 ± 39$	196 ± 24*,$
$\dot{V}_E$ (l min^{-1})	116.5 ± 16.2	73.2 ± 13.1*	108.3 ± 17.8	99.9 ± 18.3$
R	1.18 ± 0.07	1.08 ± 0.04	1.12 ± 0.09	1.14 ± 0.08
$[La]_b$ (mM)	12.0 ± 1.3	7.2 ± 2.0*	11.8 ± 1.4	12.2 ± 2.6$
$\dot{V}_{CO_2}$ (l min^{-1})	3.78 ± 0.51	2.12 ± 0.29*	3.48 ± 0.68	2.93 ± 0.36*,$

Data are presented as mean ± SD. $\dot{V}_{O_{2max}}$, maximal oxygen consumption; $\dot{w}_{max}$, maximal aerobic mechanical power; $\dot{V}_E$, minute ventilation; R, respiratory exchange ration; $[La]_b$, blood lactate concentration; $\dot{V}_{CO_2}$, carbon dioxide output; PRE, pre-bed rest values; POST, post-bed rest values.
* Significantly different from pre condition in the same posture.
$ Significantly different from corresponding upright condition.

certified gas mixtures and by means of a 3-l syringe (Hans Rudolph, Kansas City, MO, USA). f_H was monitored by means of a short-distance telemetry cardiotachometer (Polar 3000, Polar Electro, Finland).

Continuous recording of arterial pulse pressure were obtained at a fingertip on the left arm by means of a non-invasive cuff pressure recorder (Portapres, MS, Amsterdam, The Netherlands). Beat-by-beat mean arterial pressure ($\bar{P}$) was computed as the integral mean of each pressure profile, using the Beatscope software package, that is delivered with the Portapres system. Q_{st} was determined on a beat-by-beat basis by means of the Modelflow method (Wesseling et al., 1993), applied off-line to the pulse pressure profiles, using again the Beatscope software package. Beat-by-beat $\dot{Q}$ was computed as the product of single-beat Q_{st} times the corresponding single beat f_H. Each beat-by-beat $\dot{Q}$ value was then corrected for a proportionality factor (Tam et al., 2004) to account for the method's inaccuracy (Houtman et al., 1999; Azabji-Kenfack et al., 2004). To this aim, steady state $\dot{Q}$ values were obtained at rest also by means of an inert gas rebreathing device (Innocor, Innovision, Odense, Denmark). This device has been validated against thermodilution and direct Fick measurement method (Christensen et al., 2000). The calculated correction factors were then applied also at exercise, because they were demonstrated to be independent of the exercise intensity (Tam et al., 2004). A $\dot{Q}$ comparison between corrected Modelflow values and corresponding values obtained with the open circuit acetylene technique (Barker et al., 1999) showed a bias of 0.24 l min^{-1}, which was not significantly different from 0, indicating that the two methods provide the same results indeed (Tam et al., 2004). Moreover, the inter-subject variability of the two methods turned out to be the same. This indicates that the determination of $\dot{Q}$ by model flow method, after appropriate correction procedure, can adequately be employed during exercise steady state, with the significant advantage under the circumstances of the present study of reducing the duration of each submaximal work load by 1–2 min.

Blood haemoglobin concentration ([Hb]) was measured by a photometric technique (HemoCue, Sweden) on 10 μl blood samples from an earlobe. Arterial oxygen saturation (Sa_{O_2}) was measured by infrared oximetry (Siemens MicrO_2, Denvers, MA, USA). Arterial oxygen concentration (Ca_{O_2}, ml l^{-1}) was calculated as:

$$Ca_{O_2} = Sa_{O_2} \cdot [Hb] \cdot \sigma \quad (1)$$

where constant σ is the physiological oxygen binding coefficient of haemoglobin (1.34 mL g^{-1}).

$\dot{Q}a_{O_2}$ was finally obtained as the product of $\dot{Q}$ times Ca_{O_2}.

Beat-by-beat total peripheral resistance (R_p, mmHg min l^{-1}) was calculated by dividing each $\bar{P}$ value by the corresponding $\dot{Q}$ value, on the assumption that the pressure in the right atrium can be neglected as a determinant of peripheral resistance. The left ventricular stroke work (W_H) was determined as the product of $\bar{P}$ times Q_{st} and was expressed in J after appropriate unit conversion. The left ventricular power ($\dot{W}_H$) was then obtained as the product of W_H times f_H and expressed in W. Blood lactate concentration was measured by means of an electro-enzymatic method (Biosen C_line, EKF Diagnostic, Barleben, Germany) on 10 μl blood samples from an earlobe.

2.5. Statistics

Mean values are reported along with their standard deviations (SD). Absolute values before and after bed rest with the two exercise modes were compared by means of a two-way repeated analysis of variance. When applicable, a Tukey post hoc test was used to locate significant differences. The level of significance was set at $P < 0.05$ (two-tails test). For Figs. 5 and 6, the regression equation was computed with the least squares method, corrected to account for variability of both dependent and independent variables, as described by Brace (1977). The slope was compared with that of the theoretical line by means of analysis of covariance. In the same figures, values are given as mean and standard error.

3. Results

The metabolic and respiratory data obtained at maximal exercise before and after the bed rest in the two investigated postures are summarized in Table 1. $\dot{V}_{O_{2max}}$ was reduced ($P < 0.05$) after bed rest both upright and supine (on average, −38.6% and −17.0%, respectively). Although $\dot{V}_{O_{2max}}$ was not significantly different before bed rest between the two postures (upright 3.2% higher than supine), it was 30.8% higher supine than upright ($P < 0.05$) after bed rest. Accordingly, the corresponding $\dot{w}_{max}$ was reduced after bed rest in both postures (−43.1% upright, −12.2% supine, $P < 0.05$ in both cases). After bed rest, $\dot{w}_{max}$ was higher ($P < 0.05$) supine than upright (+37.4%).

Maximal $\dot{V}_{CO_2}$, lactate, $\dot{V}_E$, and R did not differ between the two postures before bed rest. Maximal $\dot{V}_{CO_2}$ was reduced by 44.0% and 15.9% after bed rest, upright and supine, respectively ($P < 0.05$ in both cases), so that after bed rest maximal $\dot{V}_{CO_2}$ was 38.4% higher supine than upright ($P < 0.05$). Maximal lactate concentration was reduced (−39.8%, $P < 0.05$) upright after bed rest due to active recovery, but no changes occurred supine (+3.1%, N.S., resting recovery). Maximal $\dot{V}_E$ was reduced upright after bed rest (−37.2%, $P < 0.05$), but not supine (−7.7%, N.S.), so that after bed rest maximal $\dot{V}_E$ was 36.5% higher supine than upright ($P < 0.05$). Maximal R was not significantly different between postures and was unaffected by bed rest.

The results of cardiovascular parameters are reported in Figs. 1 and 2. Maximal f_H was neither affected by position nor by bed rest. After bed rest, maximal Q_{st} upright was lower (−44.3%, $P < 0.05$) than before bed rest, but unchanged supine (+3.7%, N.S.), so

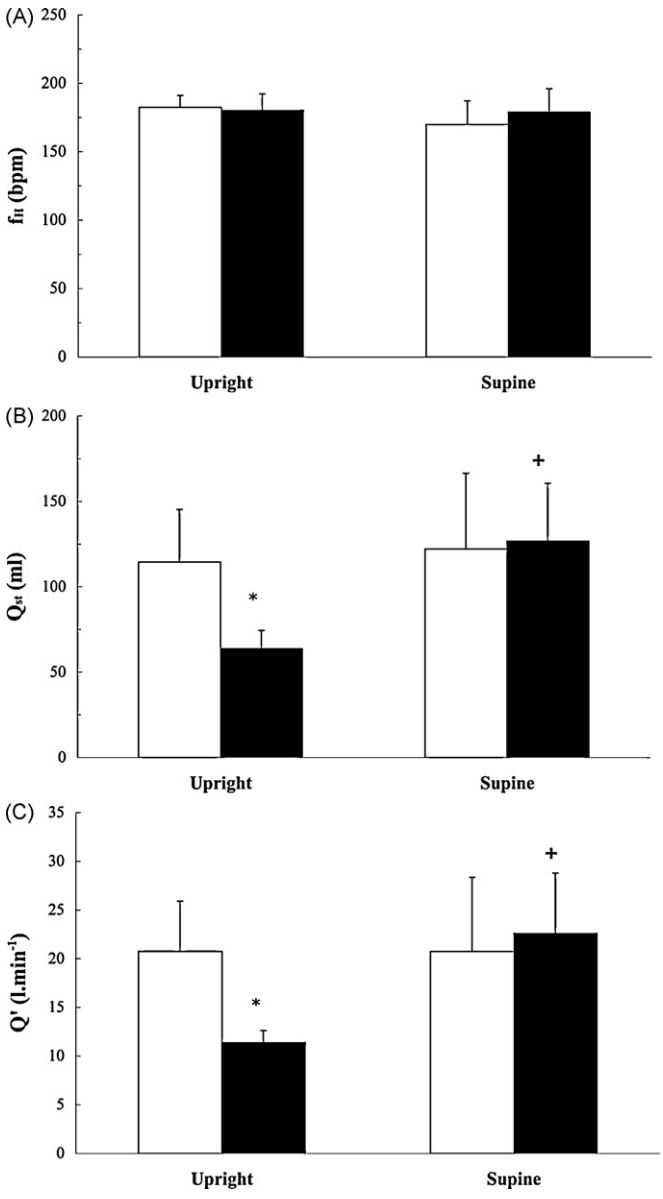

Fig. 1. Maximal heart rate (graph A), stroke volume (graph B) and cardiac output (graph C) in upright and supine posture, before (white histograms) and after (black histograms) bed rest. Vertical bars indicate SD. *Significantly different from before bed rest in the same posture; +significantly different from the corresponding upright posture.

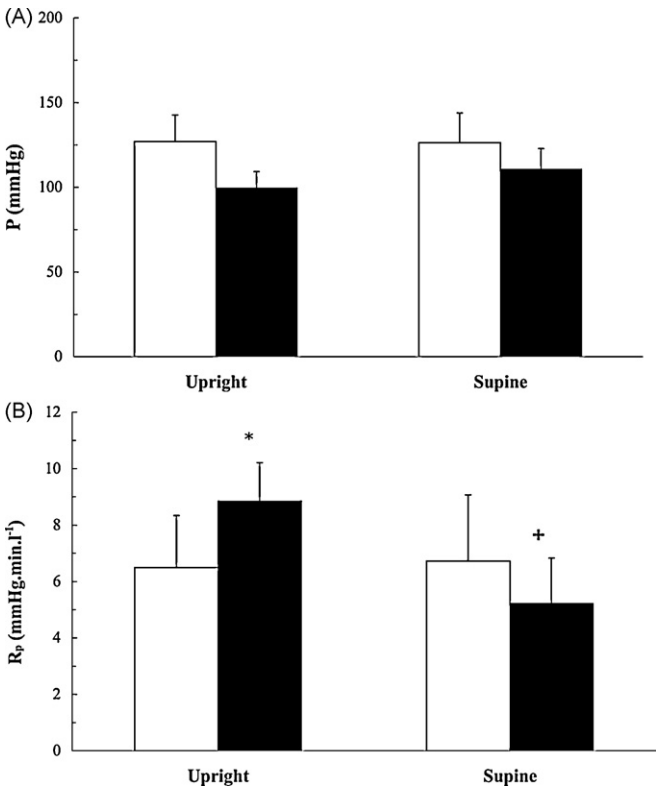

Fig. 2. Mean arterial pressure (graph A) and total peripheral resistance (graph B) at maximal exercise in upright and supine posture, before (white histograms) and after (black histograms) bed rest. Vertical bars indicate SD. *Significantly different from before bed rest in the same posture; +significantly different from the corresponding upright posture.

that after bed rest it was 98.9% higher supine than upright ($P<0.05$). As a consequence, maximal $\dot{Q}$ was decreased upright after bed rest (−45.1%, $P<0.05$), in contrast with the lack of significant changes observed supine (+9.0%, N.S.). Thus, although maximal $\dot{Q}$ before bed rest was not different between the two postures, it was higher supine than upright after bed rest (+98.4%, $P<0.05$). $\bar{P}$ did not differ between postures, and was unaffected by bed rest. R_p was the same in the two postures before bed rest (3.7% difference, N.S.). It was higher upright (+36.3%, $P<0.05$) and tended to be lower supine (−22.4%, N.S.) after bed rest than before bed rest. Therefore, after bed rest, R_p was lower supine than upright (−41.0%, $P<0.05$).

W_H and $\dot{W}_H$ are reported in Fig. 3. Bed rest reduced W_H upright (−55.7%, $P<0.05$) but not supine (−9.3%, N.S.), so that after bed rest W_H was 122.8% higher supine than upright ($P<0.05$). Since f_H was neither affected by position nor by bed rest, there was a significant decrease in $\dot{W}_H$ upright after bed rest (−56.4%, $P<0.05$), in contrast with the lack of changes supine (−5.0%, N.S.). Thus, although $\dot{W}_H$ before bed rest was not different between postures, it was higher supine than upright after bed rest (+121.5%, $P<0.05$).

The results of [Hb], Sa_{O_2} and Ca_{O_2} at $\dot{V}_{O_2max}$ are reported in Table 2. [Hb] was increased after bed rest upright (+14.3%, $P<0.05$), but not supine. Thus, although [Hb] before bed rest was not different between postures, it was lower supine than upright after bed rest (−12.5%, $P<0.05$). Sa_{O_2} during maximal aerobic exercise was neither affected by position nor by bed rest. As a consequence, Ca_{O_2} was increased after bed rest upright (+12.9%, $P<0.05$), but not supine. Thus, although Ca_{O_2} before bed rest was not different between postures, it was lower supine than upright after bed rest (−11.7%, $P<0.05$). The changes in $\dot{Q}a_{O_2}$ are reported in Fig. 4. Bed rest reduced $\dot{Q}a_{O_2}$ upright (−37.8%, $P<0.05$), but not supine (+14.8%, N.S.), so that after bed rest $\dot{Q}a_{O_2}$ was higher (+74.8%, $P<0.05$) supine than upright.

4. Discussion

To our knowledge, this was the first study in which metabolic and cardiovascular data obtained on the same subjects at maximal exercise in both the upright and supine posture, before and after long-duration bed rest, were compared. In all previous bed rest studies, $\dot{V}_{O_2max}$ was investigated in one posture only, mainly upright (Saltin et al., 1968; Stremel et al., 1976; Friman, 1979; Convertino et al., 1982; Kashihara et al., 1994; Ferretti et al., 1997; Smorawinski et al., 2001; Mekjavic et al., 2005; Capelli et al., 2006; Lee et al., 2007; Ferretti and Capelli, 2009), rarely supine (Greenleaf et al., 1989; Trappe et al., 2006). Moreover, we had the opportunity of performing $\dot{V}_{O_2max}$ tests upright on day $R+0$, actually 1 h only after the subjects stood up again at the end of the bed rest period, i.e. when the biggest $\dot{V}_{O_2max}$ changes upright are to be expected, as

Table 2
Mean values (±SD) of blood haemoglobin concentration, arterial O_2 saturation and arterial O_2 concentration.

	Upright		Supine	
	PRE	POST	PRE	POST
[Hb] ($g\,dl^{-1}$)	15.5 ± 0.8	17.7 ± 1.2*	15.0 ± 1.1	15.5 ± 0.8$
Sa_{O_2} (%)	97.7 ± 1.12	96.4 ± 0.73	96.8 ± 0.60	97.3 ± 1.32
Ca_{O_2} ($ml\,l^{-1}$)	202.4 ± 11.1	228.5 ± 15.7*	194.1 ± 15.1	201.8 ± 11.4$

Data are presented as mean ± SD. [Hb], blood haemoglobin concentration; Sa_{O_2}, arterial O_2 saturation; Ca_{O_2}, arterial O_2 concentration; PRE, pre-bed rest values; POST, post-bed rest values.
* Significantly different from pre condition.
$ Significantly different from upright condition.

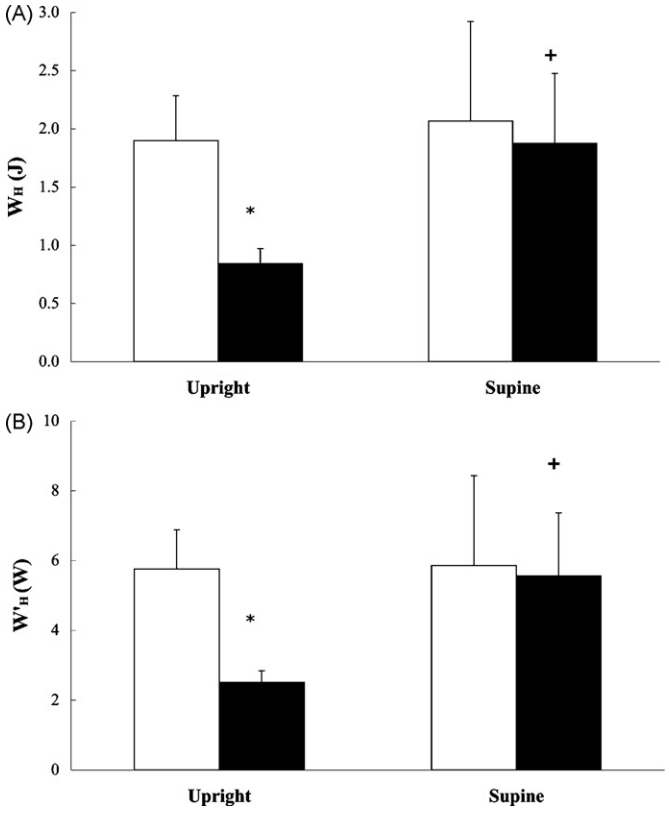

Fig. 3. Left ventricular stroke work (graph A) and power of the heart (graph B) at maximal exercise in upright and supine posture, before (white histograms) and after (black histograms) bed rest. Vertical bars indicate SD. *Significantly different from before bed rest in the same posture; +significantly different from the corresponding upright posture.

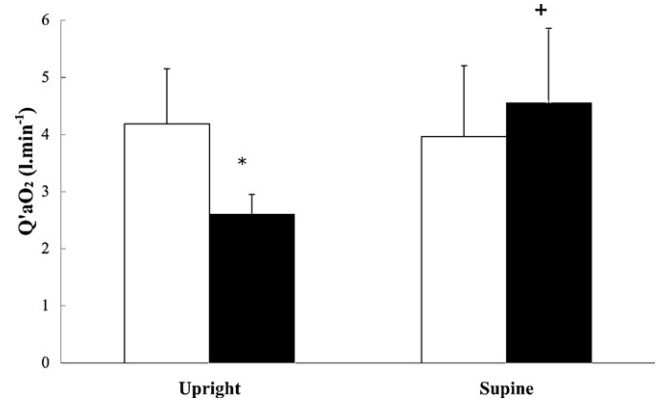

Fig. 4. Maximal systemic oxygen delivery in upright and supine posture, before (white histograms) and after (black histograms) bed rest. Vertical bars indicate SD. *Significantly different from before bed rest in the same posture; +significantly different from the corresponding upright posture.

discussed below. The results showed that after bed rest: (i) the decrease in $\dot{V}_{O_2max}$ observed upright was associated with a consensual larger decrease in maximal $\dot{Q}$ and $\dot{Q}a_{O_2}$; (ii) the decrease in $\dot{V}_{O_2max}$ observed supine was smaller than that observed upright and was associated with a lack of changes in maximal $\dot{Q}$ and $\dot{Q}a_{O_2}$; as a consequence, after bed rest; (iii) $\dot{V}_{O_2max}$ turned out lower upright than supine; (iv) the decrease in maximal $\dot{Q}$ and $\dot{Q}a_{O_2}$ observed upright was entirely related to posture. These results imply that central (cardiovascular) factors, after bed rest: (i) did not cause the observed decrease in $\dot{V}_{O_2max}$ supine; (ii) entirely explained the difference in $\dot{V}_{O_2max}$ between the two postures, and thus (iii) induced only a fraction of the overall decrease in $\dot{V}_{O_2max}$ upright.

These results have also more general implications concerning the factors determining $\dot{V}_{O_2max}$ limitation. In fact they allow experimental testing of the predictions of the multifactorial model of $\dot{V}_{O_2max}$ limitation (di Prampero and Ferretti, 1990). A quantitative analysis of the differences between postures after bed rest, carried out in the context of this model, is the object of the second part of Section 4. Here we anticipate that the present results turn out compatible with the model's prediction that cardiovascular oxygen flow, as reflected by maximal $\dot{Q}a_{O_2}$, provides some 70% of the overall $\dot{V}_{O_2max}$ limitation, at least during exercise with big muscle groups.

4.1. The origin of the $\dot{V}_{O_2max}$ decrease in supine and upright posture after bed rest

Before bed rest, $\dot{V}_{O_2max}$ did not differ significantly between postures, being 3.2% higher upright than supine. Similar postural effects on $\dot{V}_{O_2max}$ were observed by Leyk et al. (1994) ($\dot{V}_{O_2max}$ 5.5% higher upright than supine, N.S.). By contrast, others observed significantly higher $\dot{V}_{O_2max}$ values upright than supine (Saltin et al., 1976; Ray and Cureton, 1991). These authors attributed these differences in $\dot{V}_{O_2max}$ to a lower mean blood pressure gradient across the active muscles associated with lower maximal f_H and $\dot{Q}$. This would imply a reduction in muscle blood flow at maximal exercise (Cerretelli et al., 1986) and may reflect the negative gravitational gradient between heart and active muscle mass. In fact in this study, similar to Leyk's et al. (1994), the centre of rotation of the crank's axis was some 15 cm above the heart (30 cm above the body's support), perhaps less than in previous studies (data unfortunately not given in Saltin et al., 1976; Ray and Cureton, 1991), which would explain the small, non significant difference in $\dot{V}_{O_2max}$ between the two postures before bed rest in this study.

After 35 days of uninterrupted bed rest, $\dot{V}_{O_2max}$ upright was reduced by 38.6%. This is the largest $\dot{V}_{O_2max}$ decrease observed so far after bed rest of similar duration. For instance, in upright posture, Lee et al. (2007, 2009) found a 18.9% and a 16.3% decrease, in men and women, respectively, after 30 days of bed rest, whereas Mekjavic et al. (2005) observed a 17.9% decrease after 35 days of bed rest, and Ferretti et al. (1997) reported a 16.6% decrease after

42 days of bed rest. All these drops are less than a half of the one observed in the present study. The present $\dot{V}_{O_2max}$ decrease in upright posture is even larger than that observed by Capelli et al. (2006) after 90 days of bed rest (−32.4%). Perhaps the reported $\dot{V}_{O_2max}$ decrease closest to the present one, after taking into account the differences in bed rest duration, is that found by Saltin et al. (1968) after 20 days of bed rest (−28%). The larger $\dot{V}_{O_2max}$ reduction observed in this than in most previous studies may be explained by the fact that the subjects have been evaluated right on the day when they ended the bed rest ($R+0$), actually about one hour after they stood up again. By analogy, we note that the subjects investigated by Saltin et al. (1968) were studied on the day after the end of bed rest ($R+1$). By contrast, in most other studies–Lee et al. (2007, 2009) state in fact that their measures were done "immediately after" the end of bed rest without, however, giving the exact time—subjects were tested on day $R+3$ or $R+4$, allowing for a possible partial recovery of $\dot{V}_{O_2max}$ upright with respect to the very end of bed rest as a consequence of possible initial cardiovascular recovery (Spaak et al., 2005). Therefore, most studies in the past might have underestimated the impact of cardiovascular adaptation to bed rest on the $\dot{V}_{O_2max}$ measured upright at the end of bed rest. Indeed we showed that the largest $\dot{V}_{O_2max}$ decreases in upright posture at the end of bed rest are to be expected on day $R+0$.

The $\dot{V}_{O_2max}$ decrease after bed rest in upright posture has already been attributed by previous authors to the effect of cardiovascular adaptation to microgravity on blood volume distribution in that posture upon gravity resumption. In fact $\dot{V}_{O_2max}$ was found to remain unchanged during space flight (Levine et al., 1996), although the data of Trappe et al. (2006) at 85% of the maximal aerobic power suggest a slight decrease of it. Levine et al. (1996) related the keeping up of $\dot{V}_{O_2max}$ during space flight to appropriate cardiovascular adaptation to microgravity, with preserved cardiac filling. In spite of this, they observed on their subjects, who incidentally did not undergo specific exercise countermeasures during the flight, an immediate reduction of $\dot{V}_{O_2max}$ upright on the landing day. This reduction was attributed to a dramatic decrease in maximal Q_{st} that could not be compensated for by any increase in maximal f_H, resulting in a large drop in maximal $\dot{Q}$ (Levine et al., 1996). In the present study, after bed rest, maximal Q_{st} upright was reduced by 44% with no modification in maximal f_H, leading to a 45% decrease in maximal $\dot{Q}$. Thus, the decrease in maximal Q_{st} entirely explained the decrease in maximal $\dot{Q}$. Such a decrease would entail *per se* a dramatic drop of $\bar{P}$ upright: this was not observed. On one side, the lack of changes in $\bar{P}$ may be a consequence of the strong vasoconstriction in peripheral blood vessels, witnessed by the major increase in R_p, and likely mediated by an increase in sympathetic activity (Ertl et al., 2002). This last would also explain, incidentally, why the subjects had equal maximal f_H at lower $\dot{w}_{max}$ and $\dot{V}_{O_2max}$ in the upright compared to the supine posture. On the other side, muscle pump action, sustaining venous return, may also contribute to keeping up $\bar{P}$ at exercise. In supine posture, by contrast, R_p tended to be lower (−22.4%, although N.S.) after than before bed rest: we speculate that the large mass of blood displaced to the heart from very compliant lower limb veins (Belin de Chantemèle et al., 2004) might have released vasoconstriction in peripheral blood vessels, at least in most of the subjects, perhaps through stimulation of cardiopulmonary baroreceptors. The final consequence of this state of affairs is the large significant difference in R_p between the two postures after bed rest, despite the lack of difference before bed rest.

If indeed the cardiovascular system plays a major role in determining the $\dot{V}_{O_2max}$ decrease after bed rest in upright posture, this would clearly explain why the largest $\dot{V}_{O_2max}$ decrease ever observed after bed rest occurred in this study. The present diminution of maximal $\dot{Q}$ was larger than that observed after a 42-day bed rest (Ferretti et al., 1997) or even after a 90-day bed rest (Capelli et al., 2006). We measured maximal $\dot{Q}$ in the same protocol as for $\dot{V}_{O_2max}$, thus at $R+0$ instead of $R+3$ or $R+4$, as in those studies. In the same context we also note that Spaak et al. (2005) found different submaximal $\dot{Q}$ values upright at day $R+0$ from day $R+3$ after the end of a 120-day bed rest, the latter being higher than the former at the same mechanical power. Moreover, after space flight, Levine et al. (1996) observed a tendency toward a progressive increase in $\dot{V}_{O_2max}$ and maximal $\dot{Q}$ from $R+0$ to $R+2$ and $R+6$. This tendency may be a consequence of progressive restoration of circulating blood volume. Coherent with this view is the higher [Hb] observed at $R+0$ than at $R+1$ in the present study. At $R+0$, due to a 14% increase in [Hb], Ca_{O2} was increased by 13% during maximal exercise. Clearly, hypovolemia at day $R+0$ was still capable to keep [Hb] up in the present subjects, despite the likely drop in total red blood cell volume. As a consequence, maximal $\dot{Q}a_{O_2}$ upright decreased less than maximal $\dot{Q}$ (38% instead of 45%) after bed rest.

If indeed the $\dot{V}_{O_2max}$ decrease observed in upright posture was due to the reduction in maximal Q_{st}, $\dot{Q}$ and $\dot{Q}a_{O_2}$, then the hypothesis that $\dot{V}_{O_2max}$ is not to change after bed rest in supine posture because of preservation of maximal Q_{st} appears inevitable. In contrast with this hypothesis, the data show that the $\dot{V}_{O_2max}$ supine was 17.0% lower after than before bed rest. These results are in agreement with those from a previous study, which reported an 18.2% reduction of $\dot{V}_{O_2max}$ supine after 28 days of bed rest (Greenleaf et al., 1989). However, the fall of $\dot{V}_{O_2max}$ supine was not a consequence of changes in maximal $\dot{Q}$ and $\dot{Q}a_{O_2}$, because these were not different from before bed rest, in agreement with the tested hypothesis. If any, they rather tended to increase (see Figs. 2 and 4). So, other factors should be called upon to explain the observed lower $\dot{V}_{O_2max}$ supine after than before bed rest. We speculate, on the basis of the multifactorial model of $\dot{V}_{O_2max}$ limitation (di Prampero and Ferretti, 1990), that an impairment of peripheral gas flow and/or utilisation could be the concomitant factor possibly acting on another of the multiple in-series resistances along the respiratory system, and thus affecting $\dot{V}_{O_2max}$ after bed rest. Previous studies associated, at least in part, such impairment to the development of muscle hypotrophy (Ferretti et al., 1997), whose slow time course may parallel that of the slower component of the $\dot{V}_{O_2max}$ decrease induced by bed rest (Capelli et al., 2006). Lower limb muscle mass changes were not determined in the present bed rest campaign. It is noteworthy, however, that several studies reported, at the end of bed rest campaigns of comparable duration to the present one, a relative loss of lower limb muscle mass similar to that observed in the present study for $\dot{V}_{O_2max}$ supine (Berg et al., 1997, 2007; Leblanc et al., 1988).

Saltin et al. (1968) observed a decrease of Q_{st} and $\dot{Q}$ after bed rest, without modification of $\dot{V}_{O_2}$, during supine cycling at about 100 W, and suggested that an effect on myocardial function by bed rest cannot be excluded. This hypothesis was supported by the data of Perhonen et al. (2001), who showed a decreased left ventricular end diastolic volume after 2 weeks of bed rest and a decreased cardiac mass after 6 weeks of bed rest. However, an impaired heart performance would have affected maximal $\dot{Q}$ and $\dot{W}_H$. This was not the case, as demonstrated by the fact that $\dot{W}_H$ supine was unchanged after bed rest (see Fig. 3). So we tend to exclude this factor as a determinant of the $\dot{V}_{O_2max}$ decrease in supine posture in the present study.

4.2. A quantitative analysis of $\dot{V}_{O_2max}$ limitation after bed rest

The changes in $\dot{Q}a_{O_2}$ after bed rest were nil in supine posture, dramatic in upright. The $\dot{V}_{O_2max}$ reduction supine could not be attributed to cardiovascular alterations, and an impairment of

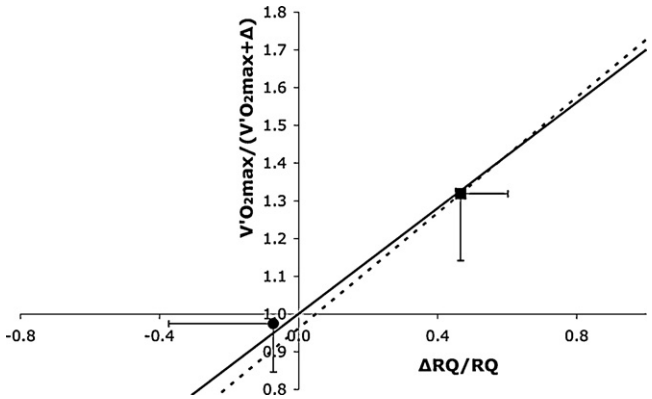

Fig. 5. The ratio between the $\dot{V}_{O_{2max}}$ supine and the $\dot{V}_{O_2max}$ upright is reported $[\dot{V}_{O_{2max}}/(\dot{V}_{O_{2max}} + \Delta)]$ as a function of the relative change in the cardiovascular resistance to oxygen flow ($\Delta R_Q/R_Q$, *x*-axis) (di Prampero and Ferretti, 1990). The continuous line, with a slope of 0.7, is theoretical and is taken from di Prampero and Ferretti (1990). The dashed line is experimental and represents the regression equation calculated from the individual data of the present study after bed rest, yielding: $y = 0.76x + 0.96$. The slope of the experimental line (0.76) was not significantly different from that of the theoretical line (0.7). The *y*-intercept of the experimental line was not significantly different from 1. Thus, the two lines resulted statistically equal. The symbols are the mean values before (circle) and after (square) bed rest. Error bars indicate standard error, expressing the variability of present mean values as compared with the general mean, represented by the theoretical line.

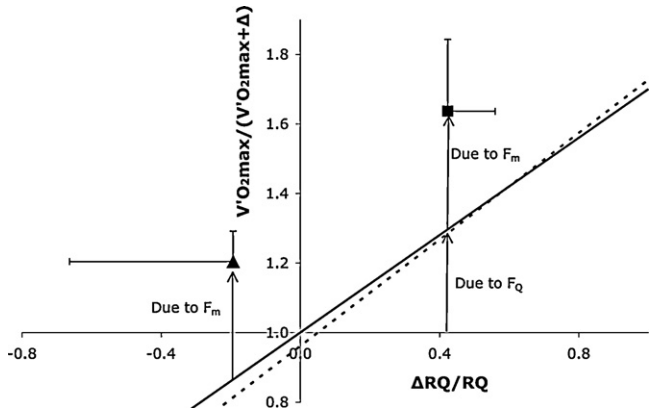

Fig. 6. The ratio between the $\dot{V}_{O_{2max}}$ after and the $\dot{V}_{O_{2max}}$ before bed rest is reported $[\dot{V}_{O_{2max}}/(\dot{V}_{O_{2max}} + \Delta)]$ as a function of the relative change in the cardiovascular resistance to oxygen flow ($\Delta R_Q/R_Q$, *x*-axis) (di Prampero and Ferretti, 1990). The continuous line, with a slope equal to 0.7, is theoretical and is taken from di Prampero and Ferretti (1990). The dashed line is the experimental line shown in Fig. 5. The symbols are the mean values in upright (square) and supine (triangle) posture. Both points lie well above the theoretical and the experimental lines. The arrows indicate how much of the $\dot{V}_{O_{2max}}$ change is due to R_Q changes (due to F_Q) and how much is due to peripheral (muscular) factors (due to F_m). Note that in supine posture mean $\Delta R_Q/R_Q$ resulted slightly negative, so that it did not explain the $\dot{V}_{O_{2max}}$ decrease supine. Error bars indicate standard error, expressing the variability of present mean values as compared with the general mean, represented by the theoretical line.

peripheral gas exchange was called upon, possibly related to the development of muscle hypotrophy, which would equally act in both postures. In the context of the multifactorial model of $\dot{V}_{O_2max}$ limitation (see Appendix A), let us define the supine posture as the normal position for a man after microgravity adaptation, and let us consider moving from supine to upright as a manoeuvre acting on the cardiovascular system only. By decreasing maximal $\dot{Q}$ and $\dot{Q}a_{O_2}$, this implies an increase in the cardiovascular resistance to oxygen flow (R_Q) (di Prampero and Ferretti, 1990). If this is so, then (i) the $\dot{V}_{O_2max}$ supine is the $\dot{V}_{O_2max}$ before the manoeuvre and the $\dot{V}_{O_2max}$ upright is that after the manoeuvre, (ii) the R_Q supine is the starting R_Q, and (iii) ΔR_Q is the change in R_Q induced acutely by the change of posture. In Fig. 5, we have plotted (see appendix) the ratio between the $\dot{V}_{O_2max}$ supine and that upright as a function of the ratio $\Delta R_Q/R_Q$. On the same figure we have reported (i) the line for $F_Q = 0.7$ (theoretical line, di Prampero and Ferretti, 1990), (ii) the mean data that refer to the effects of a change in posture from supine to upright executed after and before bed rest, and (iii) the regression line through the individual experimental points. It appears that both mean values lie very close to the theoretical line, and that the regression had a slope (F_Q) of 0.76, a value fairly close to 0.7. Thus, on one side, the induced changes in R_Q generated $\dot{V}_{O_2max}$ changes that corresponded exactly to what one could predict on the basis of the multifactorial model. On the other side, it is legitimate to conclude that the fall of $\dot{V}_{O_2max}$ when moving from supine to upright after bed rest is caused only by inadequate cardiovascular response to upright posture.

In Fig. 6, the same analysis has been carried out on the post- versus pre-bed rest data in supine and upright posture. Neither the data for supine nor those for upright lie on the theoretical line, but are placed well above it. In supine, the $\dot{V}_{O_2max}$ decrease occurred in absence of any changes in R_Q: R_Q is not a determinant of the $\dot{V}_{O_2max}$ decrease after bed rest in this posture. The vertical distance from the data point to the theoretical line is the same for both postures: the factor that caused the $\dot{V}_{O_2max}$ decrease supine after bed rest acted by the same extent upright. Since the lungs do not limit $\dot{V}_{O_2max}$ in normoxia in subjects, who even at maximal exercise operate on the flat part of the oxygen equilibrium curve (Dempsey and Wagner, 1997; Dempsey et al., 2008; Ferretti and di Prampero, 1995), the factor affecting the $\dot{V}_{O_2max}$ decrease supine after bed rest can only act on the lumped peripheral (muscular) resistance R_m. R_m reflects (i) the capillary-to-cell oxygen transfer, that is roughly proportional to muscle capillary density, and (ii) the muscle oxidative capacity, that is proportional to muscle mitochondrial volume and muscle oxidative enzyme activity (Hoppeler and Fluck, 2003; Howald and Hoppeler, 2003). Contrary to the former, the latter is largely decreased because of muscle hypotrophy and reduced mitochondrial density (Ferretti et al., 1997), whence the increase in R_m. The contribution of R_Q and R_m to the $\dot{V}_{O_2max}$ decrease upright after bed rest is shown in Fig. 6 by the two vertical arrows. R_Q provided 49% and R_m 51% of the overall $\dot{V}_{O_2max}$ reduction upright.

4.3. Limitation of the study

Lack of subjects' randomisation is a limitation of this study. In fact, recovery of impaired cardiovascular data in upright posture occurs already within 24 h after standing up again after prolonged bed rest and is aided by the performance of exercise (Engelke et al., 1996; Convertino, 2003). Previous data, however, suggested that there would have been little impairment of cardiovascular oxygen flow in supine posture after real or simulated microgravity exposure. Levine et al. (1996) clearly demonstrated lack of changes in $\dot{V}_{O_2max}$ on space flight day 8, whereas a significant reduction of $\dot{V}_{O_2max}$ appeared only in upright posture, upon return to Earth. Moreover, resting and submaximal $\dot{Q}$ were not affected in supine posture after bed rest (Sundblad et al., 2000; Spaak et al., 2005; Linnarsson et al., 2006) at least up to 60 days of bed rest. So, we took the decision of performing the supine tests at $R+1$. The observed lack of changes in $\dot{Q}$ and $\dot{Q}a_{O_2}$ after bed rest in supine posture at maximal exercise, together with the graphical analysis of Figs. 5 and 6, demonstrated that the effects of cardiovascular function impairment acted only in upright posture, thus minimizing the impact of not having been allowed to randomise tests between $R+0$ and $R+1$. On the other side, peripheral (muscular) factors, which would affect $\dot{V}_{O_2max}$ supine, are subjected to much slower recovery kinetics (Capelli et al., 2006), unable to provide visible changes in the first days after bed rest. Thus, the decision of performing the tests upright on $R+0$, and the tests supine, which are not under the

influence of orthostatic intolerance and its potential early recovery, on $R+1$, turned out to be the right choice under the present circumstances. Correction of hypovolaemia, witnessed by the decrease in [Hb] from $R+0$ to $R+1$, might have provided slightly higher $\dot{Q}$ values supine on day $R+1$ than one could have expected on day $R+0$. These postulated differences in $\dot{Q}$ would have been compensated for, however, by the concomitant opposite changes in [Hb], so that the impact of a potential overestimate of $\dot{Q}$ supine on $R+1$ would have had very small, if not altogether negligible, impact on $\dot{Q}a_{O_2}$ and $\dot{V}_{O_2max}$ supine.

4.4. Conclusions

We conclude that the 17.0% decrease in $\dot{V}_{O_2max}$ after bed rest in supine posture was not due to increased R_Q changes, because $\dot{Q}a_{O_2}$ remained unchanged. According to the multifactorial model of $\dot{V}_{O_2max}$ limitation, only R_m changes may then justify this decrease, possibly related to impaired peripheral gas exchange (reduced mitochondrial volume). On the other hand, the lower $\dot{V}_{O_2max}$ after bed rest in upright than in supine posture was entirely due to the impaired cardiovascular response upright, leading to lower $\dot{Q}a_{O_2}$ and R_Q. As a consequence, the 38.6% decrease in $\dot{V}_{O_2max}$ after bed rest in upright posture would be due to the combined effect of impaired peripheral gas exchange and altered cardiovascular response. The cardiovascular system of humans does not deteriorate in microgravity. Exercise capacity is maintained indeed, apart for the effects of muscle hypotrophy, as demonstrated by the results obtained in supine posture. However, in order to maintain system homeostasis, cardiovascular regulation adapts in such a way that, when gravity is resumed, the cardiovascular system is unable to provide an adequate response to exercise in upright posture, whence the fall in $\dot{V}_{O_2max}$ and the onset of orthostatic intolerance.

These results have some consequences on space medicine. On one side, in future microgravity experimental campaigns, exercise testing should be planned as close as possible to day $R+0$, when the greatest changes should be found. On the other side, the subject of cardiovascular countermeasures during space flight ought to be placed under a different perspective. The central question is not how to prevent cardiovascular deconditioning, but rather how to minimize the impact of gravity resumption on the cardiovascular system. Should we maintain a gravitational-like stimulus on the cardiovascular system during space flight even at the expense of some system dysfunction? LBNP appears as a useful tool under this perspective, especially when coupled with the execution of aerobic exercise (Lee et al., 2007, 2009), yet the most brilliant idea so far, although of difficult implementation, is di Prampero's twin-bike system (di Prampero, 2000). Alternatively, the European Space Agency supports the use of short-arm centrifuges, but they still require appropriate testing. In view of a flight to Mars, due to the greater impact of muscle hypotrophy with increasing time in space, cardiovascular countermeasures should however be associated with explosive and high-resistance exercise countermeasures to control muscle hypotrophy as well. In sum, the longer the space flight duration the greater is the importance of combining different interventions in designing an adequate countermeasure programme for astronauts.

Acknowledgments

This research was supported by the Swiss National Science Foundation grant 3200B0-114033 to Guido Ferretti, and an Italian Space Agency grant ASI/DCMC to Carlo Capelli and Daniela Lucini.

The Ankaran Bed Rest campaign 2008 was organized by the Italian Space Agency and by the University of Primorska, Kopar, Slovenia. Many thanks to all of the very dedicated staff at the Ankaran Hospital, Slovenia, were the bed rest was organized and the experiments took place in July and August 2008. In particular, we greatly acknowledge the cooperation of Prof. Rado Pišot and Dr. Boštian Simunič from University of Primorska. The authors are very grateful to the volunteers for their excellent dedication to the study.

Appendix A.

Each step along the oxygen cascade can be viewed as a resistance (R_i) overcome by a pressure gradient (ΔP_i). At steady state, the oxygen flow through each resistance is equal. At maximal exercise, it is given by:

$$\dot{V}_{O_2max} = \frac{\Delta P_T}{R_T} = \frac{\Delta P_i}{R_i} \tag{A1}$$

where R_T designates the overall resistance to oxygen flow, overcome by the overall pressure gradient ΔP_T. Since the resistances are arranged in-series, R_T is equal to the sum of all the individual R_i. Similarly, ΔP_T is equal to the sum of all individual ΔP_i. In humans, ΔP_T is equal to:

$$\Delta P_T = P_{IO_2} - Pm_{O_2} \tag{A2}$$

where P_{IO_2} and Pm_{O_2} designate the partial pressure of oxygen in inspired ambient air and in the mitochondria, respectively. Assuming n resistances in-series, since as a first approximation Pm_{O_2} can be set equal to 0 mmHg, we can then write:

$$\dot{V}_{O_2max} = \frac{P_{IO_2}}{R_T} = \frac{\Delta P_1}{R_1} = \frac{\Delta P_2}{R_2} = \ldots = \frac{\Delta P_{(n-1)}}{R_{(n-1)}} = \frac{\Delta P_n}{R_n} \tag{A3}$$

Several manipulations induce changes in $\dot{V}_{O_2max}$, while, as far as humans stay at sea level, no changes in ΔP_T occur. If this is so, then any change in $\dot{V}_{O_2max}$ must be entirely due to an opposite change in R_T, and thus in one or more R_i. Thus, after a given manipulation, we have:

$$\dot{V}_{O_2max} + \Delta\dot{V}_{O_2max} = \frac{P_{IO_2}}{(R_T + \Delta R_T)} \tag{A4}$$

where $\Delta\dot{V}_{O_2max}$ is the change in $\dot{V}_{O_2max}$ observed after a given manipulation has induced a given change in R_T (ΔR_T). Note thus that in Eq. (A4) $\dot{V}_{O_2max}$ corresponds to the value *before* the manipulation, whereas $\dot{V}_{O_2max} + \Delta\dot{V}_{O_2max}$ designates the value observed *after* the manipulation. Dividing Eqs. (A3) and (A4) we obtain:

$$\frac{\dot{V}_{O_2max}}{(\dot{V}_{O_2max} + \Delta\dot{V}_{O_2max})} = 1 + \frac{\Delta R_T}{R_T} \tag{A5}$$

which, because of the equivalence set in Eq. (A3), can also be written as follows:

$$\frac{\dot{V}_{O_2max}}{(\dot{V}_{O_2max} + \Delta\dot{V}_{O_2max})} = 1 + \frac{(\Delta R_1 + \Delta R_2 + \ldots + \Delta R_{(n-1)} + \Delta R_n)}{R_T} \tag{A6a}$$

or, after developing it:

$$\frac{\dot{V}_{O_2max}}{(\dot{V}_{O_2max} + \Delta\dot{V}_{O_2max})} = 1 + \frac{\Delta R_1}{R_T} + \frac{\Delta R_2}{R_T} + \ldots + \frac{\Delta R_{(n-1)}}{R_T} + \frac{\Delta R_n}{R_T} \tag{A6b}$$

In an analogous of Ohm's law, the ratio of each specific resistance i to R_T is equal to the fraction F_i of the overall limitation to $\dot{V}_{O_2max}$ provided by that resistance:

$$F_i = \frac{R_i}{R_T} \tag{A7}$$

By solving Eq. (A7) for R_T and substituting it into Eq. (A6b) for each resistance at stake, we obtain:

$$\frac{\dot{V}_{O_2max}}{(\dot{V}_{O_2max} + \Delta\dot{V}_{O_2max})} = 1 + \frac{F_1 \cdot \Delta R_1}{R_1} + \frac{F_2 \cdot \Delta R_2}{R_2} + \ldots + \frac{F_{(n-1)} \cdot \Delta R_{(n-1)}}{R_{(n-1)}} + \frac{F_n \cdot \Delta R_n}{R_n} \tag{A8}$$

A simplified solution of Eq. (A8) is obtained for the special case in which a manipulation is carried out leading to changes in one and only one resistance to oxygen flow: in this case, all the ratios on the right branch of Eq. (A8) turn out nil except one. If this very one resistance is the cardiovascular resistance R_Q, we obtain:

$$\frac{\dot{V}_{O_2max}}{(\dot{V}_{O_2max} + \Delta\dot{V}_{O_2max})} = 1 + \frac{F_Q \cdot \Delta R_Q}{R_Q} \quad (A9)$$

where F_Q is the fraction of the $\dot{V}_{O_2max}$ limitation imposed by R_Q. Eq. (A9) is the algebraic form of the line reported in Fig. 5. In fact, if we plot on the y-axis the ratio of the $\dot{V}_{O_2max}$ values before and after the manipulation (left arm of Eq. (A9), with values greater the 1 if the manipulation decreases $\dot{V}_{O_2max}$) as a function of the $\Delta R_Q/R_Q$ ratio, we would obtain e linear relationship with y-intercept equal to 1 and slope equal to F_Q (slope of lines of Fig. 5). Let's note that R_Q is the reciprocal of the product of cardiac output ($\dot{Q}$) times the oxygen transfer coefficient in the blood phase (βb), which corresponds to the average slope of the oxygen equilibrium curve. In the present study we could not determine βb due to the impossibility to measure the oxygen pressure in mixed venous blood. However, since arterial oxygen saturation (Sa_{O_2}) was invariant, changes in βb would occur only in direct proportion to changes in arterial oxygen concentration (Ca_{O_2}):

$$R_Q = \frac{1}{\dot{Q} \cdot \beta b} \approx \frac{1}{\dot{Q} \cdot Ca_{O_2}} \approx \frac{1}{\dot{Q} a_{O_2}} \quad (A10)$$

where $\dot{Q}a_{O_2}$ is the oxygen flow in arterial blood or systemic oxygen delivery. The manipulation considered for the construction of Fig. 5 is the movement from supine to upright at the end of bed rest, for which we assumed that the changes in $\dot{V}_{O_2max}$ were entirely due to the changes in $\dot{Q}a_{O_2}$.

In contrast, in Fig. 6, the experimental points do not lie on the line for $F_Q = 0.7$, but well above it, because the change in $\dot{V}_{O_2max}$ is larger than predicted from the R_Q changes only. Another resistance was increased, and this could only be the peripheral (muscular) resistance R_m (remind that the lungs do not limit $\dot{V}_{O_2max}$ in normoxia), which, as a first approximation, is inversely proportional to mitochondrial volume. So Eq. (A9) can be written as follows:

$$\frac{\dot{V}_{O_2max}}{(\dot{V}_{O_2max} + \Delta\dot{V}_{O_2max})} = 1 + \frac{F_Q \cdot \Delta R_Q}{R_Q} + \frac{F_m \cdot \Delta R_m}{R_m} \quad (A11)$$

Provided $F_Q = 0.76$, and thus $F_m = 0.24$, the ratio $\Delta R_m/R_m$ would result on average equal to 1.41 and 1.33 for U and S, respectively, whence an estimated relative decrease in mitochondrial volume of 58.6% and 57.1%.

References

Åstrand, P.O., Rodahl, K., Dahl, H.A., Stromme, S.B., 2003. Textbook of Work Physiology. Human Kinetics, Champaign, IL.

Azabji-Kenfack, M., Lador, F., Licker, M., Moia, C., Tam, E., Capelli, C., Morel, D., Ferretti, G., 2004. Cardiac output by Modelflow® method from intra-arterial and fingertip pulse pressure profiles. Clin. Sci. 106, 365–369.

Barker, R.C., Hopkins, S.R., Kellogg, N., Olfert, I.M., Brutsaert, T.D., Gavin, T.P., Entin, P.L., Rice, A.J., Wagner, P.D., 1999. Measurement of cardiac output by open-circuit acetylene technique. J. Appl. Physiol. 87, 1506–1512.

Belin de Chantemèle, E., Pascaud, L., Custaud, M.A., Capri, A., Louisy, F., Ferretti, G., Gharib, C., Arbeille, P., 2004. Calf venous volume during stand-test after a 90-day bed-rest study with or without exercise countermeasure. J. Physiol. 561, 611–622.

Berg, H.E., Eiken, O., Mekjavic, L., Mekjavic, I.B., 2007. Hip, thigh and calf muscle atrophy and bone loss after 5-week bedrest inactivity. Eur. J. Appl. Physiol. 99, 283–289.

Berg, H.E., Larsson, L., Tesch, P.A., 1997. Lower limb skeletal muscle function after 6 wk of bed rest. J. Appl. Physiol. 82, 182–188.

Brace, R.A., 1977. Fitting straight lines to experimental data. Am. J. Physiol. 233, R94–R99.

Capelli, C., Antonutto, G., Azabji-Kenfack, M., Cautero, M., Lador, F., Moia, C., Tam, E., Ferretti, G., 2006. Factors determining the time course of $\dot{V}_{O_2max}$ decay during bed rest: implications for $\dot{V}_{O_2max}$ limitation. Eur. J. Appl. Physiol. 98, 152–160.

Capelli, C., Antonutto, G., Cautero, M., Tam, E., Ferretti, G., 2008. Metabolic and cardiovascular responses during sub-maximal exercise in humans after 14 days of head-down tilt bed rest and inactivity. Eur. J. Appl. Physiol. 104, 909–918.

Cerretelli, P., Pendergast, D., Marconi, C., Piiper, J., 1986. Blood flow in exercising muscles. Int. J. Sports Med. 7, 29–33.

Christensen, P., Clemensen, P., Andersen, P.K., Henneberg, S.W., 2000. Thermodilution versus inert gas rebreathing for estimation of effective pulmonary blood flow. Crit. Care Med. 28, 51–56.

Convertino, V.A., 2003. Baroreflex-mediated heart rate and vascular resistance responses 24 h after maximal exercise. Med. Sci. Sports Exerc. 35, 970–977.

Convertino, V.A., Doerr, D.F., Eckberg, D.L., Fritsch, J.M., Vernikos-Danellis, J., 1990. Head-down bed rest impairs vagal baroreflex responses and provokes orthostatic hypotension. J. Appl. Physiol. 68, 1458–1464.

Convertino, V.A., Hung, J., Goldwater, D.J., DeBusk, R.F., 1982. Cardiorespiratory responses to exercise in middle-aged men following 10 days of bed rest. Circulation 65, 134–140.

Dempsey, J.A., McKenzie, D.C., Haverkamp, H.C., Eldridge, M.W., 2008. Update in the understanding of respiratory limitations to exercise performance in fit, active adults. Chest 134, 613–622.

Dempsey, J.A., Wagner, P.D., 1997. Exercise-induced arterial hypoxemia. J. Appl. Physiol. 87, 1997–2006.

di Prampero, P.E., 2000. Cycling on Earth, in space, on the Moon. Eur. J. Appl. Physiol. 82, 345–360.

di Prampero, P.E., Ferretti, G., 1990. Factors limiting maximal oxygen consumption in humans. Respir. Physiol. 80, 113–128.

Engelke, K.A., Doerr, D.F., Crandall, C.D., Convertino, V.A., 1996. Application of acute maximal exercise to protect orthostatic tolerance following 16 days of simulated microgravity. Am. J. Physiol. 271, R837–R847.

Ertl, A.C., Diedrich, A., Biaggioni, I., Levine, B.D., Robertson, R.M., Cox, J.F., Zuckerman, J.H., Pawelczyk, J.A., Ray, C.A., Buckey Jr., J.C., Lane, L.D., Shiavi, R., Gaffney, F.A., Costa, F., Holt, C., Blomqvist, C.G., Eckberg, D.L., Baisch, F.J., Robertson, D., 2002. Human muscle sympathetic nerve activity and plasma noradrenaline kinetics in space. J. Physiol. 538, 321–329.

Ferretti, G., Antonutto, G., Denis, C., Hoppeler, H., Minetti, A.E., Narici, M.V., Desplanches, D., 1997. The interplay of central and peripheral factors in limiting maximal O_2 consumption in man after prolonged bed rest. J. Physiol. 501, 677–690.

Ferretti, G., Capelli, C., 2009. Maximal O_2 consumption: effects of gravity withdrawal and resumption. Respir. Physiol. Neurobiol. 169S, S50–S54.

Ferretti, G., di Prampero, P.E., 1995. Factors limiting maximal oxygen consumption in humans: the effects of acute changes in ventilation. Respir. Physiol. 99, 259–271.

Ferretti, G., Girardis, M., Moia, C., Antonutto, G., 1998. Effects of prolonged bed rest on cardiovascular oxygen transport during submaximal exercise in humans. Eur. J. Appl. Physiol. 78, 398–402.

Ferretti, G., Iellamo, F., Pizzinelli, P., Azabji-Kenfack, M., Lador, F., Lucini, D., Porta, A., Narkiewicz, K., Pagani, M., 2009. Prolonged head down bed rest-induced inactivity impairs tonic autonomic regulation while sparing oscillatory cardiovascular rhythms in healthy humans. J. Hypertens. 27, 551–561.

Friman, G., 1979. Effect of clinical bed rest for seven days on physical performance. Acta. Med. Scand. 205, 389–393.

Greenleaf, J.E., Bernauer, E.M., Ertl, A.C., Trowbridge, T.S., Wade, C.E., 1989. Work capacity during 30 days of bed rest with isotonic and isokinetic exercise training. J. Appl. Physiol. 67, 1820–1826.

Hoppeler, H., Fluck, M., 2003. Plasticity of skeletal muscle mitochondria: structure and function. Med. Sci. Sports Exerc. 35, 95–104.

Houtman, S., Oeseburg, B., Hopman, M.T., 1999. Non-invasive cardiac output assessment during moderate exercise: pulse contour compared with CO_2 rebreathing. Clin. Physiol. 19, 230–237.

Howald, H., Hoppeler, H., 2003. Performing at extreme altitude: muscle cellular and subcellular adaptation. Eur. J. Appl. Physiol. 90, 360–364.

Johansen, L.B., Gharib, C., Allevard, A.M., Sigaudo, D., Christensen, N.J., Drummer, C., Norsk, P., 1997. Haematocrit, plasma volume and noradrenaline in humans during simulated weightlessness for 42 days. Clin. Physiol. Oxf. 17, 203–210.

Kashihara, H., Haruna, Y., Suzuki, Y., Kawakubo, K., Takenaka, K., Bonde-Petersen, F., Gunji, A., 1994. Effects of mild supine exercise during 20 days bed rest on maximal oxygen uptake rate in young humans. Acta. Physiol. Scand. Suppl. 616, 19–26.

Leblanc, A.D., Schneider, V.S., Krebs, J., Schonfeld, E., Evans, H.J., 1988. Calf muscle area and strength changes after five weeks of horizontal bed rest. Am. J. Sports Med. 16, 624–628.

Lee, S.M., Schneider, S.M., Boda, W.L., Watenpaugh, D.E., Macias, B.R., Meyer, R.S., Hargens, A.R., 2007. Supine LBNP exercise maintains exercise capacity in male twins during 30-d bed rest. Med. Sci. Sports Exerc. 39, 1315–1326.

Lee, S.M., Schneider, S.M., Boda, W.L., Watenpaugh, D.E., Macias, B.R., Meyer, R.S., Hargens, A.R., 2009. LBNP exercise protects aerobic capacity and sprint speed of female twins during 30 days of bed rest. J. Appl. Physiol. 106, 919–928.

Levine, B.D., Lane, L.D., Watenpaugh, D.E., Gaffney, F.A., Buckey, J.C., Blomqvist, C.G., 1996. Maximal exercise performance after adaptation to microgravity. J. Appl. Physiol. 81, 686–694.

Leyk, D., Essfeld, D., Hoffmann, U., Wunderlich, H.G., Baum, K., Stegemann, J., 1994. Postural effect on cardiac output, oxygen uptake and lactate during cycle exercise of varying intensity. Eur. J. Appl. Physiol. 68, 30–35.

Linnarsson, D., Spaak, J., Sundblad, P., 2006. Baroreflex impairment during rapid posture changes at rest and exercise after 120 days of bed rest. Eur. J. Appl. Physiol. 96, 37–45.
Mekjavic, I.B., Golja, P., Tipton, M.J., Eiken, O., 2005. Human thermoregulatory function during exercise and immersion after 35 days of horizontal bed-rest and recovery. Eur. J. Appl. Physiol. 95, 163–171.
Perhonen, M.A., Zuckerman, J.H., Levine, B.D., 2001. Deterioration of left ventricular chamber performance after bed rest: "cardiovascular deconditioning" or hypovolemia? Circulation. 103, 1851–1857.
Ray, C.A., Cureton, K.J., 1991. Interactive effects of body posture and exercise training on maximal oxygen uptake. J. Appl. Physiol. 71, 596–600.
Saltin, B., Blomqvist, C.G., Mitchell, R.C., Johnson, R.L., Wildenthal, K., Chapman, C.B., 1968. Response to exercise after bed rest and after training. Circulation 38 (Suppl. 7), 1–78.
Saltin, B., Nazar, K., Costill, D.L., Stein, E., Jansson, E., Essen, B., Gollnick, P.D., 1976. The nature of the training response: peripheral and central adaptations to one-legged exercise. Acta Physiol. Scand. 96, 289–305.
Smorawinski, J., Nazar, K., Kaciuba-Uscilko, H., Kaminska, E., Cybulski, G., Kodrzycka, A., Bicz, B., Greenleaf, J.E., 2001. Effects of 3-day bed rest on physiological responses to graded exercise in athletes and sedentary men. J. Appl. Physiol. 91, 249–257.
Spaak, J., Montmerle, S., Sundblad, P., Linnarsson, D., 2005. Long-term bed rest-induced reductions in stroke volume during rest and exercise: cardiac dysfunction vs. volume depletion. J. Appl. Physiol. 98, 648–654.
Stremel, R.W., Convertino, V.A., Bernauer, E.M., Greenleaf, J.E., 1976. Cardiorespiratory deconditioning with static and dynamic leg exercise during bed rest. J. Appl. Physiol. 41, 905–909.
Sundblad, P., Spaak, J., Linnarsson, D., 2000. Cardiovascular responses to upright and supine exercise in humans after 6 weeks of head-down tilt (−6 degrees). Eur. J. Appl. Physiol. 83, 303–309.
Tam, E., Azabji-Kenfack, M., Cautero, M., Lador, F., Antonutto, G., di Prampero, P.E., Ferretti, G., Capelli, C., 2004. Correction of cardiac output obtained by Modelflow from finger pulse pressure profiles with a respiratory method in humans. Clin. Sci. 106, 371–376.
Trappe, T., Trappe, S., Lee, G., Widrick, J., Fitts, R., Costill, D., 2006. Cardiorespiratory responses to physical work during and following 17 days of bed rest and spaceflight. J. Appl. Physiol. 100, 951–957.
Wesseling, K.H., Jansen, J.R.C., Settels, J.J., Schreuder, J.J., 1993. Computation of aortic flow from pressure in humans using a nonlinear, three-element model. J. Appl. Physiol. 74, 2566–2573.