The interplay of central and peripheral factors in limiting maximal O_2 consumption in man after prolonged bed rest

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1. The effects of bed rest on the cardiovascular and muscular parameters which affect maximal O_2 consumption ($\dot{V}_{O_2,max}$) were studied. The fractional limitation of $\dot{V}_{O_2,max}$ imposed by these parameters after bed rest was analysed.
2. The $\dot{V}_{O_2,max}$, by standard procedure, and the maximal cardiac output ($\dot{Q}_{max}$), by the pulse contour method, were measured during graded cyclo-ergometric exercise on seven subjects before and after a 42-day head-down tilt bed rest. Blood haemoglobin concentration ([Hb]) and arterialized blood gas analysis were determined at the highest work load.
3. Muscle fibre types, oxidative enzyme activities, and capillary and mitochondrial densities were measured on biopsy samples from the vastus lateralis muscle before and at the end of bed rest. The measure of muscle cross-sectional area (CSA) by NMR imaging at the level of biopsy site allowed computation of muscle oxidative capacity and capillary length.
4. The $\dot{V}_{O_2,max}$ was reduced after bed rest (−16·6%). The concomitant decreases in $\dot{Q}_{max}$ (−30·8%), essentially due to a change in stroke volume, and in [Hb] led to a huge decrease in O_2 delivery (−39·7%).
5. Fibre type distribution was unaffected by bed rest. The decrease in fibre area corresponded to the significant reduction in muscle CSA (−17%). The volume density of mitochondria was reduced after bed rest (−16·6%), as were the oxidative enzyme activities (−11%). The total mitochondrial volume was reduced by 28·5%. Capillary density was unchanged. Total capillary length was 22·2% lower after bed rest, due to muscle atrophy.
6. The interaction between these muscular and cardiovascular changes led to a smaller reduction in $\dot{V}_{O_2,max}$ than in cardiovascular O_2 transport. Yet the latter appears to play the greatest role in limiting $\dot{V}_{O_2,max}$ after bed rest (>70% of overall limitation), the remaining fraction being shared between peripheral O_2 diffusion and utilization.

The limitation to maximal O_2 consumption ($\dot{V}_{O_2,max}$) was classically attributed to cardiovascular O_2 transport (Rowell, 1974; Saltin, 1977; Blomqvist & Saltin, 1983; Ekblom, 1986). However, several other factors, such as muscle O_2 diffusion and mitochondrial oxidative capacity, have been considered as possibly limiting $\dot{V}_{O_2,max}$, particularly during exercise with small muscle groups (Kaijser, 1970; Saltin, 1977). The controversy over $\dot{V}_{O_2,max}$ limitation was recently reopened by the introduction of models which assume that each of the multiple steps of the O_2 pathway from air to mitochondria can provide a given fraction of the overall $\dot{V}_{O_2,max}$ limitation. On one side, Wagner (1996) stressed the relative role of peripheral (muscle O_2 diffusion) as opposed to central (cardiovascular) factors limiting $\dot{V}_{O_2,max}$. On the other side, a multifactorial model of $\dot{V}_{O_2,max}$ limitation, based on the O_2 cascade, was proposed (di Prampero & Ferretti, 1990).

The latter model predicts that cardiovascular O_2 transport imposes some 60–70% of $\dot{V}_{O_2,max}$ limitation in humans working with large muscle groups in normoxia. This prediction was confirmed by a systematic study of cardiovascular O_2 transport after autologous blood re-infusion,

which allowed application of the model (Turner *et al.* 1993). A simulation, supported by experimental results, allowed a quantitative estimate of the increase in the fractional limitation to $\dot{V}_{O_2,max}$ imposed by pulmonary ventilation in hypoxia (Ferretti & di Prampero, 1995). However, to our knowledge, an overall analysis of the fractional $\dot{V}_{O_2,max}$ limitation after complex adaptive phenomena, which imply changes in both cardiovascular O_2 transport at maximal exercise and morphometric muscle oxidative and diffusive capacities, simultaneously determined on the same experimental subjects, is currently missing in the literature. The only exception, a study on exercise after altitude acclimatization (Cerretelli & Ferretti, 1990), was neither performed nor interpreted within the context of $\dot{V}_{O_2,max}$ limitation.

A unique opportunity for a global analysis of the factors which limit $\dot{V}_{O_2,max}$ in normoxia came from the undertaking of a long-term head-down tilt bed rest study by the European Space Agency and by the Centre National d'Etudes Spatiales, France, in 1994. In fact bed rest leads to remarkable reductions in $\dot{V}_{O_2,max}$ due to an impairment of both cardiovascular and muscular functions (for a review see Fortney, Schneider & Greenleaf, 1996). Such functional deteriorations imply changes in several resistances to O_2 flow along the O_2 transfer system. It was thus argued that, based on these changes, the multifactorial model would allow a discrimination of the relative roles of peripheral (muscular) and central (cardiovascular) factors in determining the $\dot{V}_{O_2,max}$ reduction after bed rest.

In the present study, a detailed analysis of the effects of prolonged bed rest on both the cardiovascular and muscular parameters expected to affect $\dot{V}_{O_2,max}$, simultaneously assessed on a homogeneous group of subjects, is reported. The relative weight of these parameters in determining the $\dot{V}_{O_2,max}$ reduction after bed rest and the fraction of $\dot{V}_{O_2,max}$ limitation imposed by them have been analysed by means of the multifactorial model.

METHODS

Study design

After approval by the local ethical committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Toulouse I, France), the study was conducted on seven healthy young males, who had previously given their written informed consent. At the beginning of the study, they were 28 ± 1 years old, $1{\cdot}76 \pm 0{\cdot}01$ m tall and $74{\cdot}0 \pm 3{\cdot}3$ kg in weight. All experiments were carried out at the Hôpital Purpan, Toulouse, France, except for NMR imaging, which was performed at the Hôpital Rangueil, Toulouse.

The study consisted of three phases: (1) baseline control experiments before bed rest; (2) a 42-day head-down tilt (−6 °) bed rest period without countermeasures: no deviations from the lying position were permitted, and neither exercise nor muscle contraction tests were allowed during this period; (3) final experiments after bed rest. These included exercise testing on day 4 during recovery, whereas the muscle biopsy had to be taken on day 37 of the bed-rest period, in order to avoid interference with other concomitant protocols after bed rest. NMR imaging of the legs was thus carried out on day 37 of bed rest.

Maximal O_2 consumption

Individual $\dot{V}_{O_2,max}$ was determined during graded exercise on the bicycle ergometer (Ergometrics 800-S, Ergoline, Germany). The oxygen uptake ($\dot{V}_{O_2}$) at the metabolic steady state was measured at rest and during exercises of increasing intensities. Starting from 100 and 50 W before and after bed rest, respectively, power was progressively augmented by steps of 50 W, reduced to 25 W as the expected individual maximum power was approached. The duration of each work load was 5 min. Successive work loads were separated by 5 min recovery intervals, during which time blood samples (20 μl) were obtained from an ear lobe at 1, 3 and 5 min for determination of blood lactate concentration ($[La]_b$) by means of an electro-enzymatic method (ESAT 6661 Lactat, Eppendorf, Germany).

During the fifth minute of each work load, expired gas was collected into Douglas bags and subsequently analysed for gas composition and volume. A paramagnetic O_2 analyser (Oxynos 1-C, Leybold Haereus, Germany), an infrared CO_2 analyser (LB-2, Leybold Haereus) and a dry gas meter (Singer DTM 15, USA) were employed. Steady-state $\dot{V}_{O_2}$ was computed by standard expiratory mass balance equations. CO_2 output ($\dot{V}_{CO_2}$), expired ventilation ($\dot{V}_E$) and the gas exchange ratio were also calculated.

Individual $\dot{V}_{O_2,max}$ was established from the plateau attained by the relationship between $\dot{V}_{O_2}$ and power above a given power. If such a plateau was not observed, subsidiary criteria for $\dot{V}_{O_2,max}$ establishment were: (1) a lack of increase in heart rate (HR) between two successive work loads ($\Delta HR < 5$ min^{-1}); (2) gas exchange ratio values higher than 1·1; (3) $[La]_b$ values higher that 10 mM. The HR was measured by electrocardiography (Elmed, ETM, Germany). The minimum power requiring an energy expenditure equal to $\dot{V}_{O_2,max}$ ($\dot{w}$ at $\dot{V}_{O_2,max}$) was defined as the mechanical power ($\dot{w}$) at the crossing point of the line describing the $\dot{V}_{O_2}$ *vs.* power relationship and the plateau defining $\dot{V}_{O_2,max}$. The overall net mechanical efficiency of exercise was calculated as the reciprocal of the slope of the relationship between $\dot{V}_{O_2}$ and power.

Cardiovascular O_2 transport

Maximal cardiac output ($\dot{Q}_{max}$) was determined during the same procedure as for the determination of $\dot{V}_{O_2,max}$ from HR and stroke volume measurements (Q_H). The latter was measured by means of the pressure-pulse contour method (Antonutto, Girardis, Tuniz & di Prampero, 1995). To this end, continuous monitoring of the arterial blood pressure profile was obtained by means of a Finapres device (Ohmeda, USA). The photo-plethysmographic cuff of the Finapres was applied to the middle phalanx of the middle finger of the right hand. The arterial pressure profiles were recorded on magnetic tape, digitalized by an A/D converter and subsequently analysed by a computer (Acqknowledge® III for the MP 100 WS, Biopac Systems Inc., Goleta, CA, USA).

Calibration of the area described by the arterial blood pressure profiles at rest was performed against mean stroke volume values obtained from cardiac output determinations using the one-step CO_2 rebreathing method (Farhi, Nesarajah, Olszowka, Metildi & Ellis, 1976). The Ground Respiratory Monitoring System (Innovision, Odense, Denmark) equipped with a mass spectrometer (Balzers, Liechtenstein) and a personal computer was used. The rebreathing mixture contained 35% oxygen, so that alveolar–capillary gas equilibration was maintained throughout the entire rebreathing manoeuvre.

At exercise, the algorithm provided by Antonutto *et al.* (1995) was used for signal calibration. This algorithm relies on the calibration of the pulse-pressure area performed at rest. Application of this algorithm to the computation of cardiac output during exercise was validated against the CO_2 rebreathing method for a range of cardiac output values comprised between 4 and 22 l min^{-1} (G. Antonutto, C. Capelli, P. E. di Prampero, M. Girardis, D. Pendergast & P. Zamparo, unpublished observations).

At rest and immediately after the end of the highest work load, an 80 μl arterialized blood sample was obtained from the ear lobe, previously made hyperaemic by means of an ointment (Trafuril, Ciba Geigy, Switzerland), and immediately analysed (Ciba Corning 280 blood gas system, USA) for arterialized blood pH (pH_a), haemoglobin concentration [Hb] and arterialized O_2 and carbon dioxide partial pressures (P_{a,O_2} and P_{a,CO_2}, respectively). Arterial O_2 saturation (S_{a,O_2}) was measured continuously by finger tip infrared oxymetry (Pulsox-5, Minolta, Japan). Arterialized oxygen concentration (C_{a,O_2}) at the exercise steady state was then calculated as the product of [Hb], S_{a,O_2} and physiological O_2 binding coefficient of haemoglobin (1·34 ml g^{-1}). Arterial O_2 delivery ($\dot{Q}_{a,O_2}$) at maximal exercise was calculated as the product of $\dot{Q}_{max}$ times C_{a,O_2}. Mixed venous O_2 concentration ($C_{\bar{v},O_2}$) was computed by the Fick equation. The Q_H was calculated as the ratio of $\dot{Q}_{max}$ to HR.

Muscle biopsies

Muscle biopsies were taken under local anaesthesia from the same location and depth on the mid portion of the vastus lateralis of the dominant thigh, 29 days before bed rest and during the 37th day of bed rest, using a Weil–Blakesley conchotome (Henriksson, 1979). Two to three centimetres separated the two sites of the repeated incisions on the same thigh. Samples were divided for histochemistry, biochemistry and electron microscopy.

Histochemical analysis

A 5 mm thick block was mounted in an embedding medium (Tissue-TEK ACT compound, Miles Inc. Diagnostics Division, Elkhart, IN, USA), frozen in isopentane, pre-cooled in liquid nitrogen, and stored at −80°C until analysis. Serial transverse sections (10 μm) were cut on a microtome at −30°C and were stained for myosin adenosinetriphosphatase (ATPase) activity (Brooke & Kaiser, 1970). After pre-incubation at differing pH values (4·3, 4·5) in acid buffer (acetic acid, 50 mM) with 25 mM $CaCl_2$ for 4 min at 25°C, the ATPase reaction was carried out in a buffer (pH 9·4) with 18 mM $CaCl_2$ and 2·7 mM ATP at 37°C for 20 min. Based on observed differences in pH lability of the myosin ATPase activity of the isomyosins in the different fibres, muscle fibres were classified into three major types (I, II A, and II B) and an intermediate type (II AB). Fibre type composition is expressed as the number of fibres of each type relative to the total number of fibres. Measurements were made on approximately 350 fibres in each section. A minimum of fifty fibres of each fibre type was used for the calculation of the fibre cross-sectional area by a computerized planimetry system coupled to a digitizer. Mean fibre area was expressed as the cumulative area divided by the number of fibres. Type II AB mean fibre area was not computed, due to their small number. Capillaries were stained using the ATPase technique after pre-incubation at pH 4·0. Capillary density and number of capillaries per fibre were determined as described by Andersen & Henriksson (1977).

Enzyme assays

Muscle homogenates were prepared in 0·3 M phosphate buffer, containing 0·05% bovine serum albumin (pH 7·7). They were frozen and thawed three times to disrupt the mitochondrial membrane. Hexokinase (HK, EC 2.7.1.1), lactate dehydrogenase (LDH, EC 1.1.1.27) and 3-hydroxyacyl-CoA dehydrogenase (HAD, EC 1.1.1.35) were fluorimetrically determined as previously described by Lowry & Passonneau (1973). Citrate synthase (CS, EC 4.1.3.7) was measured by the method of Srere (1969) using 5-5′-dithiobis(2-nitrobenzoic acid). Enzyme activities are expressed as micromoles of substrate per minute per gram of protein.

Morphometry

A fraction of the muscle tissue was processed for electron microscopy by fixing in a 6·25% solution of glutaraldehyde as previously described (Hoppeler, Mathieu, Krauer, Claassen, Armstrong & Weibel, 1981). Transverse ultrathin sections (60–90 nm) were cut with an LBK ultramicrotome from two tissue blocks randomly chosen from each muscle. For analysis of the mitochondria, lipid droplets and myofibrils, forty micrographs per muscle were taken on 35 mm film with a Philips 300 electron microscope at a final magnification of × 24 000. The volume densities of interfibrillar and subsarcolemmal mitochondria, and of myofibrils were determined with a systematic sampling procedure in consecutive frames of twenty square mesh grids. The reference space was the total fibre volume. The mean total volume density of mitochondria was calculated as the sum of the mean interfibrillar and subsarcolemmal mitochondrial volume densities for each muscle. Point counting was performed with a grid C 16 (144 test points). All stereological variables were estimated according to standard procedures (Weibel, 1979).

Muscle cross-sectional area

Muscle cross-sectional area (CSA) was obtained by NMR imaging (Siemens Somatom Impact 1.5 T, Erlangen, Germany) of the right and left thighs. Ten axial scans were performed (10 mm thickness, repetition time 700 ms, echo time 17 ms) at intervals equal to 1/10 of femur length. For this study, only the CSA at 5/10 of femur length was used, as it corresponds to the level where the biopsy was taken. Each film was digitally scanned (StudioScan II, Agfa Inc.) at a resolution of 150–185 dpi, and the files obtained were processed by using NIH Image 1.52 on an Apple Duo 230 computer. Contours of the anterior muscle groups (including the sartorius muscle) were digitized semi-manually, and the CSAs were obtained by converting the results of a pixel-counting routine according to a reference ruler. The CSAs of both thighs were analysed and a mean CSA was calculated. In order to repeat scans at the same individual femur lengths, appropriate anatomical markers were used.

Muscle oxidative capacity (mitochondrial volume) was calculated by multiplying the volume density of total mitochondria by the volume of a 1 cm-thick slice of muscle of known CSA around the biopsy site. Capillary length was calculated by multiplying capillary density by the volume of the same 1 cm-thick slice of muscle, assuming a factor for capillary tortuosity of 1·25. The assumption was made that all muscles of the thigh were equally affected by bed rest: hence the structural changes observed in muscle vastus lateralis were considered representative for all thigh muscles. This procedure and this assumption were described and criticized in detail in Conley, Kayar, Rösler, Hoppeler, Weibel & Taylor (1987).

Statistical analysis

The Wilcoxon ranked test was used to test the significance of intra-group differences (before and after bed rest). The level of significance was set at $P < 0{\cdot}05$. All data are expressed as means ± S.E.M. Linear regressions were calculated by means of the least square method.

Table 1. Maximal oxygen consumption and related parameters before and after bed rest

Parameter	Unit	Before	After	P(2 tails)	Change (%)
Body mass	kg	74·0 ± 3·25	72·7 ± 3·17	n.s.	−1·7
$\dot{V}_{O_2,max}$	l min^{-1}	2·83 ± 0·204	2·36 ± 0·129	<0·01	−16·6
$\dot{V}_{O_2,max}$	ml min^{-1} kg^{-1}	38·90 ± 3·719	32·98 ± 2·581	<0·01	−15·2
$\dot{w}$ at $\dot{V}_{O_2,max}$	W	229 ± 17·8	186 ± 11·3	<0·01	−18·7
$[La]_b$	mM	12·0 ± 0·57	11·2 ± 0·68	n.s.	−6·7
$\dot{V}_{CO_2,max}$	l min^{-1}	3·49 ± 0·227	3·00 ± 0·163	<0·01	−14·1
$\dot{V}_{E,BTPS}$	l min^{-1}	113·8 ± 9·11	108·0 ± 6·80	n.s.	−5·1
Gas exchange ratio	unitless	1·24 ± 0·019	1·27 ± 0·023	n.s.	+2·4

$\dot{V}_{O_2,max}$, maximum oxygen consumption; $\dot{w}$, mechanical power; $[La]_b$, blood lactate concentration; $\dot{V}_{CO_2,max}$, maximum CO_2 output; $\dot{V}_{E,BTPS}$, pulmonary ventilation at body temperature and pressure, when saturated with water vapour. Data are given as means ± S.E.M.

RESULTS

Maximal oxygen consumption

Submaximal oxygen consumption ($\dot{V}_{O_2}$) was linearly related to mechanical power in all subjects. At any submaximal power, $\dot{V}_{O_2}$ was the same before and after the bed rest period. Overall net mechanical efficiency of submaximal exercise was 0·262.

The metabolic and respiratory data obtained at maximal exercise before and after the bed rest are summarized in Table 1. Absolute $\dot{V}_{O_2,max}$ and $\dot{V}_{CO_2,max}$ were greatly reduced after bed rest (−16·6%, $P < 0·01$ and −14·1%, $P < 0·01$, respectively) but were attained at the same $[La]_b$, gas exchange ratio and $\dot{V}_E$ as in the control condition. Since body mass was unaffected by bed rest, the same changes were observed for specific $\dot{V}_{O_2,max}$ (−15·2%, $P < 0·01$). Accordingly, the $\dot{w}$ at $\dot{V}_{O_2,max}$ was greatly diminished after bed rest (−18·7%, $P < 0·01$).

Cardiovascular oxygen transport

The cardiovascular and blood gas data are reported in Table 2. The maximal HR was the same before and after bed rest. Thus, the remarkable reduction in $\dot{Q}_{max}$ (−30·8%) was the result of a dramatic decrease in Q_H. The [Hb] was also significantly reduced after bed rest (−8·6%). Since S_{a,O_2} was unchanged, a reduction in C_{a,O_2} obviously followed. These changes resulted in a huge decrease in $\dot{Q}_{a,O_2}$ at maximal exercise (−35·5%). The decrease (although not significant) in $C_{\bar{v},O_2}$ after bed rest was an inevitable consequence of the greater decrease in $\dot{Q}_{max}$ than in $\dot{V}_{O_2,max}$. After bed rest, the same pH_a and P_{a,CO_2} values at maximal exercise as in the control condition were found.

Muscle structure and function

The effects of bed rest on the percentage distribution and cross-sectional areas of fibres are shown in Fig. 1*A* and *B*, respectively. Fibre type distribution remained unchanged after bed rest. Fibre cross-sectional areas were reduced after

Table 2. Cardiovascular and haematological variables at maximal exercise before and after bed rest

Parameter	Unit	Before	After	P(2 tails)	Change (%)
HR	beats min^{-1}	194 ± 1·5	194 ± 2·3	n.s.	0·0
Q_H*	ml	123 ± 13·2	85 ± 7·94	< 0·01	−30·9
$\dot{Q}_{max}$*	l min^{-1}	23·7 ± 2·42	16·4 ± 1·44	< 0·01	−30·8
[Hb]	g l^{-1}	173 ± 5·3	157 ± 4·9	< 0·01	−8·6
S_{a,O_2}	unitless	0·96 ± 0·008	0·97 ± 0·004	n.s.	+1·0
C_{a,O_2}	ml l^{-1}	222·2 ± 6·88	204·7 ± 6·20	< 0·01	−7·9
pH_a	pH units	7·254 ± 0·0159	7·229 ± 0·0427	n.s.	−0·3
P_{a,CO_2}	mmHg	28·9 ± 1·66	26·3 ± 2·00	n.s.	−9·0
P_{a,O_2}	mmHg	90·2 ± 2·91	99·0 ± 3·44	n.s.	+9·8
$\dot{Q}_{a,O_2}$*	l min^{-1}	5·38 ± 0·654	3·47 ± 0·481	< 0·01	−35·5
$C_{\bar{v},O_2}$	ml l^{-1}	100·0 ± 39·3	62·5 ± 29·8	n.s.	−37·5

HR, heart rate; Q_H, stroke volume of the heart; $\dot{Q}_{max}$, maximal cardiac output; [Hb], blood haemoglobin concentration; S_{a,O_2}, arterial oxygen saturation; C_{a,O_2}, arterialized oxygen concentration; pH_a, arterialized blood pH; P_{a,CO_2}, arterialized carbon dioxide partial pressure; P_{a,O_2}, arterialized oxygen partial pressure; $\dot{Q}_{a,O_2}$, oxygen delivery at maximal exercise; $C_{\bar{v},O_2}$, mixed venous oxygen concentration. * Values for which $n = 5$.

Table 3. Muscle morphometry and muscle enzyme activities, as from muscle vastus lateralis biopsy specimens, before and after bed rest

	Morphometry	
	Before	After
Volume density of interfibrillar mitochondria (%)	4·59 ± 0·29	3·80 ± 0·23*
Volume density of subsarcolemmal mitochondria (%)	0·54 ± 0·09	0·48 ± 0·11
Volume density of total mitochondria (%)	5·13 ± 0·36	4·28 ± 0·31*
Volume density of intracellular lipid droplets (%)	0·77 ± 0·08	0·70 ± 0·10
Volume density of myofibrils (%)	75·8 ± 1·6	77·1 ± 1·0
	Muscle enzymes	
	Before	After
Hexokinase (μmol min^{-1} (g protein^{-1})	3·1 ± 0·1	3·5 ± 0·3
Lactate dehydrogenase (μmol min^{-1} (g protein^{-1})	762 ± 123	750 ± 151
3-Hydroxyacyl-CoA-dehydrogenase (μmol min^{-1} (g protein^{-1}))	26·1 ± 2·8	23·2 ± 2·4*
Citrate synthase (μmol min^{-1} (g protein^{-1}))	46·5 ± 7·1	42·2 ± 5·9

* Statistically significantly different ($P < 0·05$).

bed rest (4779 ± 396 *vs.* 4083 ± 152, 6091 ± 244 *vs.* 5768 ± 311, and 5199 ± 443 *vs.* 4292 ± 210 μm^2 for type I, type II A, and type II B fibres, respectively). This reduction (−15 and −17 % for type I and II B fibres, respectively) did not reach statistical significance ($P = 0·06$).

Neither capillary per fibre ratio (2·58 ± 0·04 *vs.* 2·50 ± 0·06) nor capillary density (504 ± 28 *vs.* 461 ± 27 mm^{-2}) was significantly affected by bed rest. The stereological variables are presented in Table 3. No change occurred in the volume density of lipids and myofibrils. In contrast, the volume density of interfibrillar and total mitochondria was decreased by 17 %. Muscle enzyme activities are also reported in Table 3. Exposure to bed rest induced a significant decrease (−11 %) in 3-hydroxyacyl-CoA dehydrogenase activity. The change in citrate synthase activity (−9 %) was not significant. The activities of lactate dehydrogenase and hexokinase remained unchanged compared with the control values.

Muscle cross-sectional area

The mean CSA of the extensor muscle groups at mid-femur before and after bed rest was 87·3 ± 12·1 cm^2 and 75·6 ± 8·8 cm^2, with a decrease of −13·4 % ($P < 0·005$). The muscle oxidative capacity of the 1 cm-thick slice of thigh musculature around the biopsy site was 4·5 ± 1·1 and 3·2 ± 0·6 cm^3 before and after bed rest, respectively, the

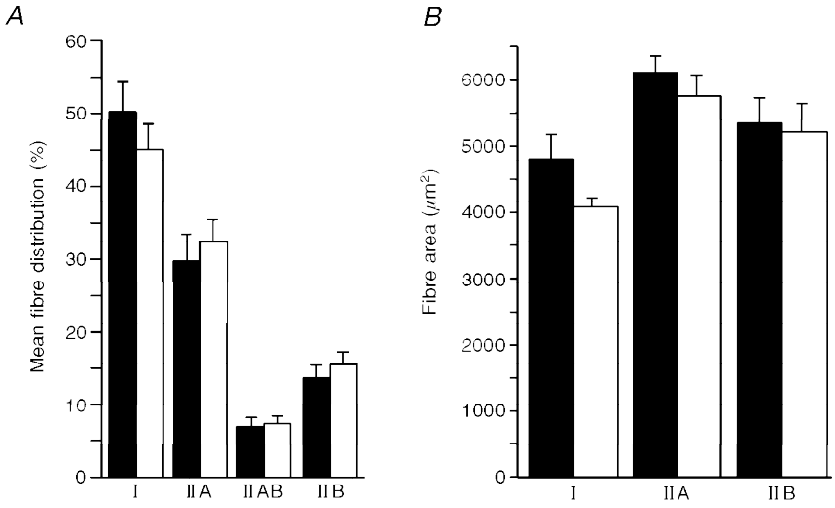

Figure 1. Muscle fibre types before and after bed rest

A, fibre type distribution; *B*, fibre type cross-sectional area. Data before (■) and after (□) bed rest for muscle fibre types.

decrease amounting to 28·5% ($P < 0·01$). The total capillary length in the same slice of thigh muscles was 44·2 ± 3·5 and 34·4 ± 0·9 km before and after bed rest, respectively, the latter value being 22·2% lower than the former ($P < 0·05$).

DISCUSSION

The $\dot{V}_{O_2,max}$ decrease observed in this study is in the upper range of changes reported in previous studies (for a review see Fortney *et al.* 1996). The fact that after bed rest the $\dot{V}_{O_2,max}$ and the mechanical power decreased by the same amount suggests that the decrease in $\dot{V}_{O_2,max}$ occurred within the active muscle mass. This allows application of the multifactorial model of $\dot{V}_{O_2,max}$ limitation in the interpretation of the numerous adaptive phenomena at the cardiovascular and muscular levels, which contributed to the decrease in $\dot{V}_{O_2,max}$ after bed rest. These phenomena are first analysed separately, and then considered in a global context, in an attempt to estimate their relative role in limiting $\dot{V}_{O_2,max}$ after bed rest.

Cardiovascular oxygen transport

The decrease in $\dot{Q}_{max}$ after bed rest, which is greater than reported in previous studies with shorter bed rest duration, seems entirely due to a decrease in Q_H, while HR remained unchanged (Fig. 2). This is in agreement with previous studies (Saltin, Blomqvist, Mitchell, Johnson, Wildenthal & Chapman, 1968; Sullivan *et al.* 1985). The decrease in Q_H may, at least in part, be a consequence of a reduced blood volume resulting from a drop of both red cell and plasma volume. The former seems reflected by the lower [Hb] after bed rest; the latter was obviously not measured at maximal exercise, but at rest, in the course of the same bed rest study on the same subjects, others found it to decrease by 9·3% after bed rest (Johansen *et al.* 1997). The decrease in Q_H, associated with a drop in mean arterial blood pressure, is indicative of a reduced capacity by the heart to produce mechanical work at maximal exercise. This is consistent with a reduction in cardiac size observed after prolonged bed rest (Saltin *et al.* 1968).

The observed reduction in C_{a,O_2} is probably due to the decreased [Hb]. This is a common finding after bed rest, resulting from the loss of red cell mass, the origin of which is still controversial (Fortney *et al.* 1996). The combined changes in $\dot{Q}_{max}$ and in C_{a,O_2} indicate a dramatic decrease in $\dot{Q}_{a,O_2}$, which appears to be a major determinant of the reduction in $\dot{V}_{O_2,max}$, as discussed below.

Muscle ultrastructure and enzyme activities

The stereological analysis showed a large decrease in the volume density of mitochondria (−17%), but no change in the myofibrillar volume density. These results are opposite to those observed on animals subjected to hindlimb suspension, showing an invariant or increased volume density of mitochondria associated with a preferential loss of contractile myofibrillar proteins (Desplanches, Favier, Sempore & Hoppeler, 1991). After bed rest, the decrease in mitochondrial volume density was not accompanied by changes in muscle capillarity. These results also are in contrast with earlier observations in unloaded rats, which show an increased capillary density, implying a reduction in diffusion distances and a more efficient oxygen and substrate delivery to muscle fibres (Desplanches *et al.* 1991).

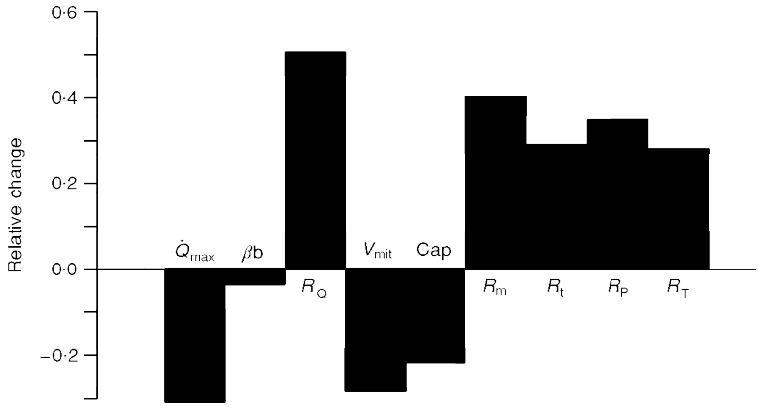

Figure 2. The determinants of maximal oxygen consumption after bed rest

$\dot{Q}_{max}$, maximal cardiac output; βb, blood oxygen transport coefficient; R_Q, cardiovascular resistance to oxygen flow; V_{mit}, muscle oxidative capacity; Cap, capillary length; R_m, mitochondrial resistance to oxygen flow; R_t, tissue diffusive resistance to oxygen flow; R_P, lumped peripheral resistance to oxygen flow ($R_t + R_m$); R_T, total resistance to oxygen flow. Data are given relative to the values before bed rest, assuming $R_t = R_m$. The predicted maximal oxygen consumption ($\dot{V}_{O_2,max}$) is 2·225 l min^{-1} for R_t and R_m in series and 2·226 l min^{-1} for R_t and R_m in parallel. These are calculated by means of the multifactorial model, from the value before bed rest and the changes in resistance shown in this figure. The measured $\dot{V}_{O_2,max}$ from this study is 2·36 ± 0·129 l min^{-1}.

This cannot be postulated for the present study. Assuming that no interstitial oedema occurred, as discussed below, the finding of a reduction in total muscle CSA and muscle fibre CSA with no changes in capillary density and in capillary/fibre ratio suggests that a net loss of muscle capillaries (i.e. of total capillary length or surface available for exchange processes) occurred during bed rest.

The changes in volume density of mitochondria are consistent with the changes observed in biochemical markers of the oxidative metabolism such as CS (−9%) and HAD (−11%) activities. The results for CS are qualitatively similar to previous reports in humans after bed rest and lower limb unloading (Hikida, Gollnick, Dudley, Convertino & Buchanan, 1989; Berg, Dudley, Hather & Tesch, 1993).

With respect to the glycolytic potential, HK and LDH activities were unchanged, consistent with the results of a 30 day bed rest study (Hikida *et al.* 1989).

Muscle fibre types and cross-sectional areas

The fibre-type populations of the vastus lateralis muscle are similar to those of a healthy young non-athletic population (see Gollnick, Armstrong, Saubert, Piehl & Saltin, 1972, Howald, 1982), determined using the same technique. The percentage of type II AB fibres observed in the present study appears to be less than is usually reported for human skeletal muscle from the analysis of myosin heavy chain expression in single muscle fibre (Larsson & Moss, 1993; Sant'Ana Pereira, Wessels, Nijtmans, Moorman & Sargeant, 1995). This discrepancy may be the consequence of the different technique used in these studies: in fact in the present study some type II AB fibres may have been classified as type II B fibres, because co-expression of type II A and type IIB myosin heavy chains can go undetected with the histochemical technique.

After thirty-seven days of bed rest, the histochemical analysis revealed no change in the percentage type distribution of fibres, consistent with earlier reports using another human model of unilateral lower limb unloading during twenty-eight or fourty-two days (Hather, Adams, Tesch & Dudley, 1992; Berg *et al.* 1993). These results disagree with previous animal studies, which led to a reduced motor activity and mechanical unloading via hindlimb unweighting (Thomason & Booth, 1990). In rats, it was demonstrated that in slow-twitch postural muscles, such as the soleus, a shift towards faster contractile properties occurred after unweighting, whereas little change was found in fast-twitch muscles (McDonald & Fitts, 1995). In fact, atrophied soleus muscles were characterized by changes in the myosin isoform distribution (an increase in fast heavy chain isoforms) and by the expression of a new myosin heavy chain II D (Takahashi, Wada & Katsuda, 1991).

The changes in cross-sectional area of both type I and II B fibres were just above the limits of statistical significance, because one subject did not show muscle atrophy at all. The possibility of sampling error implicit in fibre type distribution based on a single sample site cannot be excluded (Lexell & Taylor, 1989). Yet the apparent decline in fibre CSA is in good agreement with the results from a previous 30-day bed rest study (Hikida *et al.* 1989).

The present CSA reduction of the quadriceps muscle compares well with the 11·9 and 12% decrease after 5 and 6 weeks of unilateral limb suspension, respectively (Hather *et al.* 1992; Ploutz-Snyder, Tesch, Crittenden & Dudley, 1995). Similarly, a 12% decrease in the quadriceps CSA was reported after 1 month bed rest (Berry, Berry & Manelfe, 1993). The present data thus agree with the observed reduction in type I and II B fibre cross-sectional areas. This being the case, fibre type atrophy would appear as the main determinant of muscle atrophy, without being masked by the occurrence of interstitial oedema, as observed in animal studies (Kandarian, Boushel & Schulte, 1991).

The observed reduction in muscle CSA, and thus in muscle mass, implies a greater decrease in muscle oxidative capacity (−28·5%) than in volume density of muscle mitochondria (−16·6%). Such a decrease is comparable only to that observed on extreme altitude climbers following altitude acclimatization (Hoppeler *et al.* 1990).

$\dot{V}_{O_2,max}$ limitation after bed rest

On the model employed

The present analysis of the factors which limit $\dot{V}_{O_2,max}$ after bed rest, is carried out in the context of the multifactorial model of $\dot{V}_{O_2,max}$ limitation, which is described in detail elsewhere (di Prampero & Ferretti, 1990). We thus re-consider only the general principles utilized in this case. The model is an application of the O_2 conductance equation to maximal exercise. The flow of O_2 from ambient air to mitochondria is considered to be driven by O_2 pressure gradients against numerous resistances in series. Five resistances (R) or conductances ($G = 1/R$) of physiological interest are identified, related to: (i) alveolar ventilation (G_v or R_v); (ii) alveolar–capillary O_2 transfer (G_L or R_L); (iii) cardiovascular O_2 transport (G_Q or R_Q); (iv) tissue O_2 transfer (G_t or R_t); and (v) tissue O_2 utilization (G_m or R_m). The two last peripheral resistances can be considered either in series ($R_P = R_t + R_m$) or in parallel ($G_P = G_t + G_m$). Since the fractional limitation of $\dot{V}_{O_2,max}$ in normoxia imposed by G_v and G_L seems negligible (di Prampero & Ferretti, 1990; Ferretti & di Prampero, 1995), the present analysis focuses on G_Q, G_t and G_m only. Of these, G_Q is equal to the product of $\dot{Q}_{max}$ and the O_2 transfer coefficient of blood, which corresponds to the mean slope of the O_2 equilibrium curve $((C_{a,O_2} - C_{\bar{v},O_2})(P_{a,O_2} - P_{\bar{v},O_2})^{-1})$. In the computation of G_Q, $P_{\bar{v},O_2}$ was estimated from $C_{\bar{v},O_2}$ by means of the oxygen status algorithm (Siggaard-Andersen & Siggaard-Andersen, 1990). G_t and G_m are considered to vary proportionally to the changes in muscle capillary length and muscle oxidative capacity, respectively (Weibel, Taylor & Hoppeler, 1992).

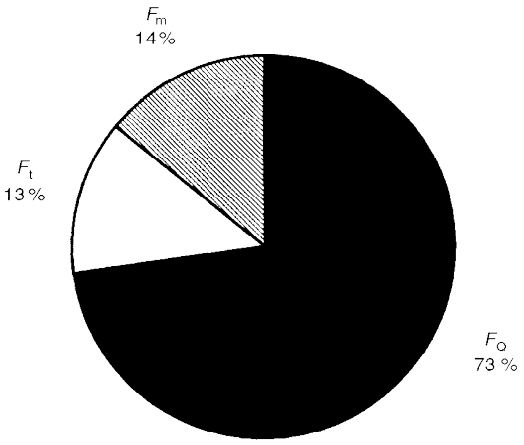

Figure 3. Factors limiting maximal oxygen consumption after bed rest

Fractional limitation to maximal oxygen consumption imposed by cardiovascular oxygen transport (F_Q), peripheral oxygen diffusion (F_t) and muscle oxidative capacity (F_m).

The approach

Since the overall O_2 pressure gradient is unchanged, the decrease in $\dot{V}_{O_2,max}$ after bed rest results only from an increase in the overall resistance to O_2 flow. This is a consequence of: (i) the dramatic impairment of O_2 delivery, as indicated by the decrease in both $\dot{Q}_{max}$ and [Hb] (Table 2), with consequent increase in R_Q; (ii) the marked drop in muscle oxidative capacity, with consequent increase in R_m; and (iii) the decrease in muscle capillary length, suggesting a greater R_t. The effects of bed rest on $\dot{V}_{O_2,max}$ have been predicted from the calculated relative changes in R_Q, R_t and R_m after bed rest, and compared with the measured ones. The results of this analysis are summarized in Fig. 2. The fractional limitation of $\dot{V}_{O_2,max}$ after bed rest is shown in Fig. 3. It appears that R_Q, the contribution of which to the change in total resistance is predominant, plays the greatest role in limiting $\dot{V}_{O_2,max}$ also after bed rest ($> 70\%$). The mean measured $\dot{V}_{O_2,max}$ value is not significantly different from the predicted one.

Since the proportion to which R_t and R_m combine to give R_P cannot be determined, the changes in R_t and R_m reported in Fig. 2 were calculated postulating $R_t = R_m$ before bed rest (di Prampero & Ferretti, 1990). This postulate is tested in Fig. 4, in which the effects on the predicted $\dot{V}_{O_2,max}$ after bed rest of various combinations of R_m and R_t values leading to the R_P value before bed rest are analysed. Figure 4 suggests that: (i) the differences in predicted $\dot{V}_{O_2,max}$ are very small from one extreme ($R_t = R_P$) to the other ($R_m = R_P$); (ii) it makes no difference to assume R_t and R_m are in series or in parallel; and (iii) the closest prediction to the actually measured $\dot{V}_{O_2,max}$ is obtained when $R_t = R_P$, despite the possibility that bed rest had greater effects on R_m than on R_t. This suggests that the peripheral resistance to O_2 flow

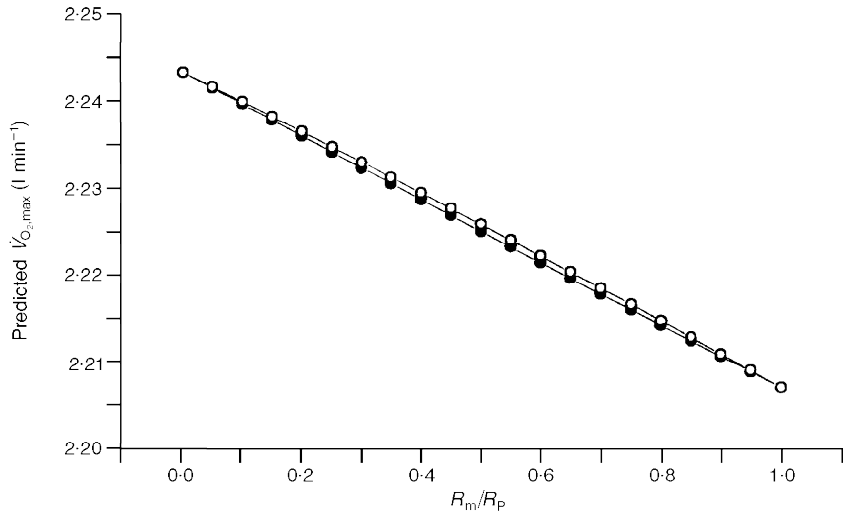

Figure 4. Theoretical analysis of the effects of the peripheral resistance to oxygen flow on maximal oxygen consumption

Predicted maximal oxygen consumption ($\dot{V}_{O_2,max}$) values are plotted as a function of theoretical ratios of mitochondrial (R_m) to lumped peripheral (R_P) resistance to oxygen flow. Two conditions are hypothesized: diffusive resistance (R_t) and mitochondrial resistance (R_m) in series (●) or R_m and R_t in parallel (○).

may be essentially due to diffusive resistances rather than mitochondrial resistances.

Factors limiting maximal oxygen consumption after bed rest

The results of Figs 2 and 3 suggest that the model used in this study is a fairly good predictor of the changes in $\dot{V}_{O_2,max}$ ensuing from complex adaptive phenomena, such as long-term bed rest. The slightly, although not significantly, lower prediction may be attributed to the neglect of the small role which lung resistances play in limiting $\dot{V}_{O_2,max}$ in normoxia. In fact, taking these resistances into account would have reduced the effects of changes in R_Q and R_P on $\dot{V}_{O_2,max}$, with predicted $\dot{V}_{O_2,max}$ values closer to the measured ones.

Figures 2 and 3 show that the $\dot{V}_{O_2,max}$ observed after bed rest results from the new equilibrium attained by the various limiting steps, each with its size and its fractional limiting role. If cardiovascular O_2 transport was the only factor limiting $\dot{V}_{O_2,max}$, as proposed in the past (Rowell, 1974; Saltin, 1977; Blomqvist & Saltin, 1983; Ekblom, 1986), the resulting changes in $\dot{V}_{O_2,max}$ would have had to be proportional to the changes in G_Q or in $\dot{Q}_{a,O_2}$. This is not so: although this analysis supports the contention that cardiovascular O_2 transport is the main determinant of $\dot{V}_{O_2,max}$ during normoxic exercise with big muscle groups, the decrease in $\dot{V}_{O_2,max}$ after bed rest was much smaller than that in $\dot{Q}_{a,O_2}$. In fact phenomena occurring at other (muscle) levels along the O_2 transfer system also contribute significantly to limit $\dot{V}_{O_2,max}$.

Conclusions

Prolonged bed rest induced a significant decrease in: (i) maximal cardiac output and haemoglobin concentration, with consequent reduction in O_2 delivery; (ii) muscle oxidative capacity and oxidative enzyme activities; and (iii) muscle capillary length, limiting the surface available for peripheral O_2 diffusion. The final result was a smaller reduction in $\dot{V}_{O_2,max}$ than in cardiovascular O_2 transport. These findings are indicative of an equilibrium between central and peripheral determinants of $\dot{V}_{O_2,max}$ limitation, even though cardiovascular O_2 transport still appears as the major factor limiting $\dot{V}_{O_2,max}$ after bed rest ($>70\%$ of the overall limitation). At the muscle level, greatest importance may be attributed to O_2 diffusion. Yet an overall analysis of muscle biopsy data reveals specific effects of thigh muscle unloading on indices related to aerobic metabolism.

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