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Capillary rarefaction during bed rest is proportionally less than fibre atrophy and loss of oxidative capacity

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Abstract

Background Muscle disuse from bed rest or spaceflight results in losses in muscle mass, strength and oxidative capacity. Capillary rarefaction may contribute to muscle atrophy and the reduction in oxidative capacity during bed rest. Artificial gravity may attenuate the negative effects of long-term space missions or bed rest.

The aim of the present study was to assess (1) the effects of bed rest on muscle fibre size, fibre type composition, capillarization and oxidative capacity in the vastus lateralis and soleus muscles after 6 and 55 days of bed rest and (2) the effectiveness of artificial gravity in mitigating bed-rest-induced detriments to these parameters.

Methods Nineteen participants were assigned to a control group (control, $n = 6$) or an intervention group undergoing 30 min of centrifugation ($n = 13$). All underwent 55 days of head-down tilt bed rest. Vastus lateralis and soleus biopsies were taken at baseline and after 6 and 55 days of bed rest. Fibre type composition, fibre cross-sectional area, capillarization indices and oxidative capacity were determined.

Results After just 6 days of bed rest, fibre atrophy ($-23.2 \pm 12.4\%$, $P < 0.001$) and reductions in capillary-to-fibre ratio (C:F; 1.97 ± 0.57 *vs*. 1.56 ± 0.41, $P < 0.001$) were proportional in both muscles as reflected by a maintained capillary density. Fibre atrophy proceeded at a much slower rate between 6 and 55 days of bed rest ($-11.6 \pm 12.1\%$ of 6 days, $P = 0.032$) and was accompanied by a 19.1% reduction in succinate dehydrogenase stain optical density ($P < 0.001$), without any further significant decrements in C:F (1.56 ± 0.41 vs. 1.49 ± 0.37, $P = 0.459$). Consequently, after 55 days of bed rest, the capillary supply–oxidative capacity ratio of a fibre had increased by 41.9% ($P < 0.001$), indicating a capillarization in relative excess of oxidative capacity. Even though the heterogeneity of capillary spacing (Log_RSD) was increased after 55 days by 12.7% ($P = 0.004$), tissue oxygenation at maximal oxygen consumption of the fibres was improved after 55 days bed rest. Daily centrifugation failed to blunt the bed-rest-induced reductions in fibre size and oxidative capacity and capillary rarefaction.

Conclusions The relationship between fibre size and oxidative capacity with the capillary supply of a fibre is uncoupled during prolonged bed rest as reflected by a rapid loss of muscle mass and capillaries, followed at later stages by a more than proportional loss of mitochondria without further capillary loss. The resulting excessive capillary supply of the muscle after prolonged bed rest is advantageous for the delivery of substrates needed for subsequent muscle recovery.

Keywords Bed rest; Atrophy; Skeletal muscle; Artificial gravity; Capillarization; Oxidative capacity

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Introduction

Disuse from bed rest, immobilization and spaceflight is associated with reductions in muscle size, strength and oxidative capacity and is the consequence of a net protein breakdown in these conditions.[1] The disuse-induced muscle wasting is more pronounced in postural muscles, such as the calf musculature, compared with non-postural muscles, such as the vastus lateralis muscle.[2] Strikingly, this muscle atrophy can be seen after just a few days of bed rest[3,4] with further reductions in muscle size occurring at a slower rate as bed rest continues.[5]

Besides muscle atrophy, bed rest could potentially also lead to accelerated fatigability, given that there is often a shift towards faster fibre type[6] and a reduction in VO_2max.[7] Although exaggerated fatigability has been reported after disuse,[8] this is not seen in all immobilization studies,[9] where there may even be no significant reduction in muscle fatigue resistance during a series of repeated shortening contractions in the presence of a higher reliance on glycolytic metabolism.[10] Although the fatigue resistance of a muscle or motor unit is positively related to its oxidative capacity,[8] blockage of functional capillaries has shown that also the capillary bed is an important determinant of muscle fatigue resistance.[11]

The capillary bed is not only important for the delivery of oxygen but also delivery of energy substrates and removal of heat and metabolic waste products.[12] The absence of a significant relationship between the capillary supply of a fibre and its oxidative capacity indicates that fibre size is probably a more important determinant of muscle capillarization than oxygen demand.[13] This is also reflected by the correlation of capillarization with fibre size in both human and rodent muscles.[13] Further supporting the coupling between capillarization and fibre size is the similar time course of hypertrophy and angiogenesis during an overload stimulus[14] and the observation in humans that the hypertrophic response is less when the muscle has a low capillary density (CD).[15]

It is therefore possible that capillary rarefaction during bed rest contributes to the observed muscle fibre atrophy. Whereas in non-disused muscle, contractile activity will elicit a rise in blood flow that enhances endothelial shear stress required to maintain the microvasculature in skeletal muscle,[12] the little contractile activity and consequently fewer and shorter episodes of elevated blood flow to the muscle during disuse will lead to arterial remodelling[16] and via reduced sheer stress most likely also result in capillary rarefaction. However, so far there are no indications whether the coupling between capillary supply to a fibre with its metabolic type and size changes during bed rest, nor of the time course of such changes.

If significant decrements in fibre size and fibre oxidative capacity, and capillary rarefaction occur after just 6 days of bed rest, it emphasizes the importance of early preventative interventions in spaceflight and hospitalization. To assess changes in the capillary supply to a muscle, both capillary-to-fibre ratio (C:F) and CD are important indices of capillarization. Where C:F is independent of fibre size and a reduction in this parameter is indicative of capillary rarefaction (assuming fibre number is not changed), CD is influenced by fibre size and is a reflection of the diffusion capacity of the capillary bed of the muscle tissue.

One potential countermeasure to the unwanted effects of muscle unloading is artificial gravity, which has been shown in pilot studies to attenuate reductions in fibre cross-sectional area (FCSA), isometric strength of the knee extensors and flexor muscles.[17] These observations recently led the European Space Agency (ESA) and the National Aeronautics and Space Administration (NASA) to design the Artificial Gravity Bed Rest Study (AGBRESA) to determine the beneficial effects of daily centrifugation on multiple physiological, psychological and behavioural parameters. As part of AGBRESA, the objectives of the present study were to assess (1) the effects of bed rest on muscle fibre size, fibre type composition, capillarization and oxidative capacity at an early time point (6 days) and after 55 days of bed rest and (2) the effectiveness of artificial gravity in mitigating bed-rest-induced decrements in these parameters. We hypothesized that (1) bed rest results in a rapid proportional reduction in fibre size and capillary rarefaction, although there is a proportionally larger loss of oxidative capacity, and (2) that these adverse adaptations to bed rest are attenuated by daily artificial gravity.

Methods

Study design

Twenty-four healthy subjects (16 men, 8 women, 33 ± 9 years; 175 ± 9 cm; 74 ± 10 kg) participated in the AGBRESA study. This was undertaken by the German Aerospace Centre, the ESA and NASA, at the DLR, Cologne, Germany, in two campaigns with 12 participants each in 2019. The objectives of the AGBRESA study were to determine the efficacy of 30 min daily artificial gravity in the form of continuous (cAG) and intermittent centrifugation (iAG) as a countermeasure to the adverse effects of immobilisation and disuse (*Figure* 1). Subjects underwent physical and psychological testing to assess their suitability to be included in the study. The study subjects were randomly assigned to groups for the first campaign, whereas for campaign 2 assignment aimed at demographic balancing of groups, as three women and one man dropped out and were subsequently replaced during the second campaign (n = 8 per group). No significant group × time interactions were found when iAG and cAG

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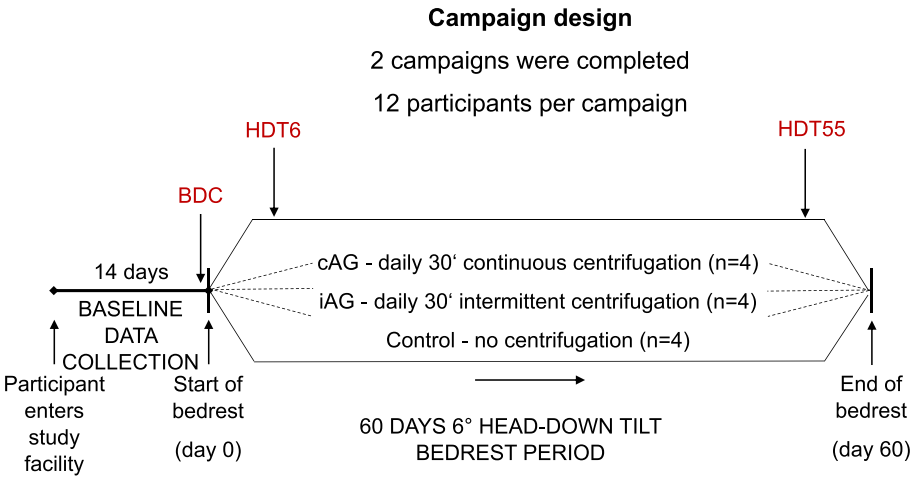

Figure 1 A graphical representation of the campaign design. The campaign was repeated twice, each with 12 participants, but due to issues with tissue freezing, histological analysis was not completed in all participants at all time points. Biopsies were taken at BDC (baseline), HDT6 and HDT55 (Day 6 and 55 head-down tilt bed rest, respectively).

groups were compared, so these groups were pooled for further analysis. Further details of the participants' daily routines in the AGBRESA study have been published by Frett et al.[18]

Biopsy acquisition

Vastus lateralis and soleus muscle biopsies (approximately 150 mg) were taken from 21 subjects using a rongeur (4 mm diameter) under sterile conditions at baseline (BDC), Day 6 and Day 55 or 57 of head-down tilt bed rest (HDT6 and HDT55/57). Samples were acquired after skin disinfection and local anaesthesia with lidocaine. Biopsies were mounted on cork with Tissue-Tek O.C.T. Compound (Sakura, Torrance, CA, USA) in orientation for transverse sections, frozen in liquid nitrogen and stored at −80°C. Sections (10 μm) were cut using a cryostat (Leica CM 3050S, Leica Biosystems, Wetzlar, Germany), mounted on adhesion slides and stored at −80°C until staining. Due to issues with tissue freezing, histological analysis was completed on samples from 19 people for the vastus lateralis (control n = 6, AG n = 13) and 18 people for the soleus (control n = 6, AG n = 12).

Fibre type and capillary staining and analysis

To identify fibre type, capillary locations and fibre outlines, one slide from each sample was left to defrost for 10 min and then blocked for 1 h with 10% goat serum (Vector Laboratories, CA, USA) in phosphate-buffered saline (PBS). Slides were incubated for 2 h with BA-D5 (1:100; specific for myosin heavy chain type I), BF-35 (1:100; all isoforms except for myosin heavy chain type IIx; Developmental Studies Hybridoma Bank, IA, USA) and rabbit polyclonal anti-dystrophin primary antibody (1:100; Abcam, ab15277) in blocking solution to identify Type I, IIa and fibre boundaries, respectively. After three 5-min PBS washes the slide was incubated for 1 h in secondary antibodies anti-mouse Alexa Fluor 555 IgG2b for type I (1:500), anti-mouse Alexa Fluor 488 IgG1 for type IIa (1:500), goat anti-rabbit cross-adsorbed Alexa Fluor 350 IgG (H + L) (1:500) for dystrophin and *Ulex europaeus* Agglutinin I fluorescein (1:250) for capillaries (Thermo Fisher Scientific, MA, USA). After 3 × 5 min washes in PBS, slides were mounted with Vectashield Antifade Mounting Medium (H-1000; Vector Laboratories, CA, USA).

Images of the vastus lateralis and soleus muscles were taken using a fluorescent microscope (Zeiss Imager Z2, Zeiss, Germany) at 10× magnification (*Figure* 2A and 2B), and images were analysed with DTect (https://ora.ox.ac.uk/objects/uuid:6d128833-4c00-46bd-b7aa-10145e5091b9). Fibre boundaries were determined semi-automatically using thresholding and allowing for manual removal of non-specific staining. Fibres were identified manually as follows: Type I fibres stained positive for BA-D5, and Type IIa were positive for BF-35, but not BA-D5 and Type IIx showed no staining with either antibody. Capillaries were manually identified by positive staining for *Ulex europaeus* Agglutinin I. Using these data, fibre cross-sectional a FCSA, fibre type composition, CD and C:F were determined. In addition, capillary domains were calculated, areas of tissue surrounding each capillary delineated by equidistant boundaries from neighbouring capillaries,[12] to determine the local capillary-to-fibre ratio (LCFR), which is the sum of the fractions of each domain which overlap the fibre, capillary fibre density (CFD: LCFR divided by the FCSA) and the logarithmic standard

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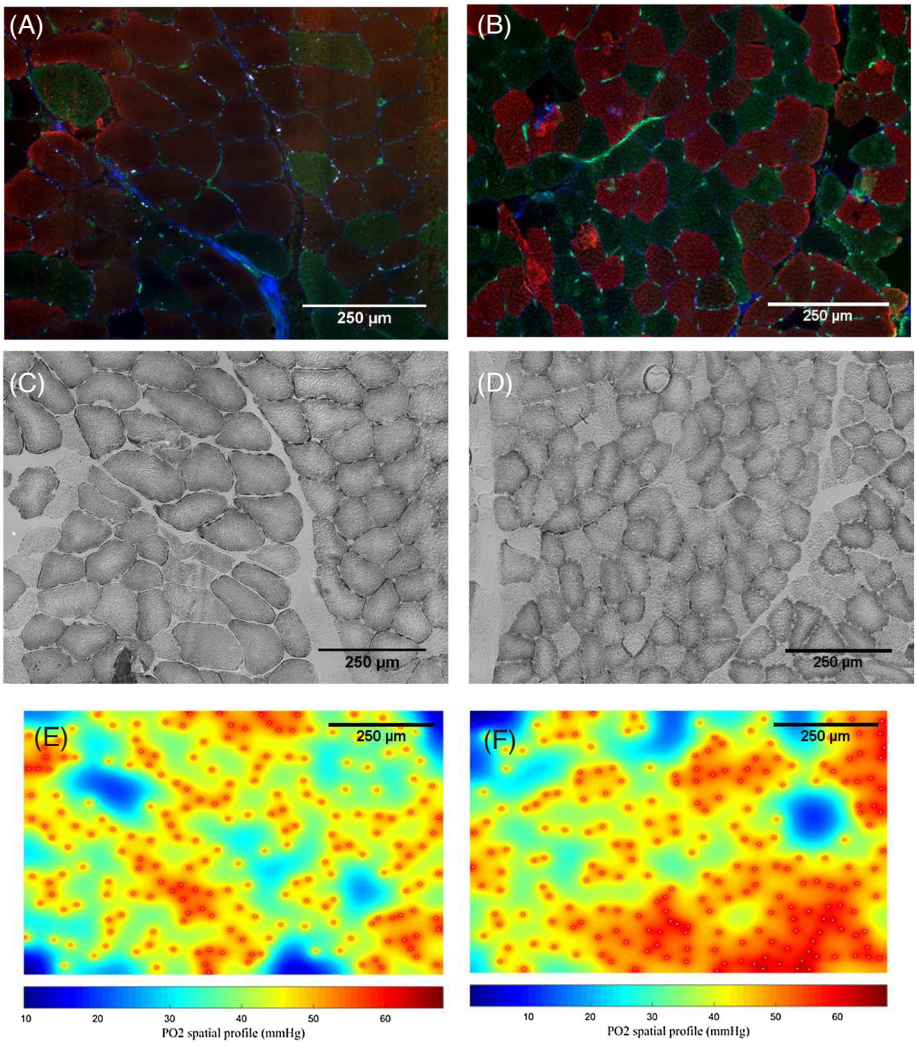

Figure 2 Serial sections of biopsies stained for (A and B) fibre type composition and capillaries, (C and D) succinate dehydrogenase (SDH) and (E and F) indicating partial pressure of oxygen (PO_2) estimate spatial profiles in vastus lateralis muscle biopsies generated by the oxygen transport modeller based on (A) and (C), respectively, at baseline (BDC) and after 55 (HDT55) days of head-down tilt bed rest. (A), (C) and (E) are taken from serial sections of BDC, (B), (D) and (F) are taken from serial sections of HDT55. (A and B) Red: Type I; green: Type IIa; unstained: Type IIx; and green dots: capillaries.

deviation of the radius of the capillary domains (Log_RSD), an index of the heterogeneity of capillary spacing. A minimum of 80 fibres were analysed per sample.

Succinate dehydrogenase staining and analysis

Serial slides were stained for succinate dehydrogenase (SDH) activity as described previously[19] (*Figure* 2C and 2D). The optical density of the SDH stain at 660 nm gives a quantitative indication of the maximal rate of oxygen consumption of muscle fibres.[19] Filters with a known optical density were imaged to create a calibration curve for each section to adjust for variation in background staining and lighting between sections. ImageJ was used to determine the optical density (SDH-OD) of the stain (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, MD, USA). Integrated SDH activity (SDH-INT) was calculated by multiplying the SDH-OD of each fibre by its FCSA.

Muscle oxygenation

Estimates of the average oxygen partial pressure (PO_2) within muscle sections were calculated with 'Oxygen transport modeler'[20] (*Figure* 2E and 2F). Model assumptions include estimates of capillary radius (1.8×10^{-4} cm), concentration of oxygen bound to myoglobin (10.2×10^{-3} mL O_2 mL^{-1}) and O_2 solubility (3.89×10^{-5} mL O_2 mL^{-1} $mmHg^{-1}$) and diffusivity (1.73×10^{-7} cm^2 s^{-1}). The average VO_2max of fibres from each sample was calculated using the SDH-INT, as follows[21]:

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$$VO_2max(\text{fibre})\ (\text{L kg}^{-1}\ \text{min}^{-1}) = 0.672 \times \text{SDH-INT}$$

The average VO_{2max} of fibres was then converted to maximal oxygen demand in mL O_2 mL^{-1} s^{-1} and fed into the model.

Statistics

Data are presented for each fibre type, but also for the weighted average of all fibres. Data were analysed with IBM SPSS Version 27. Shapiro–Wilk tests were used to determine whether data were normally distributed. Due to the prevalence of non-matching soleus and vastus lateralis muscles, an analysis of variance was used with a linear mixed-effects model with muscle type, time point and fibre type as repeated variables. In addition to main effects, two-way interactions were considered. Effects and interactions were considered significant at $P < 0.05$. Bonferroni post hoc tests were applied to determine differences between time points and fibre types.

Results

cAG versus iAG

No significant group × time interactions were found when iAG and cAG groups were compared for FCSA (P = 0.902), fibre type composition (P = 0.782), CD (P = 0.545), C:F (P = 0.835), Log_RSD (P = 0.113), SDH-OD (P = 0.440) and LCFR/SDH-INT (P = 0.219). Therefore, these groups were pooled for further analyses.

Sex differences

There were no significant sex × group interactions for FCSA (P = 0.127), fibre type composition (P = 0.718), CD (P = 0.766), C:F (P = 0.280), Log_RSD (P = 0.054), SDH-OD (P = 0.662) and LCFR/SDH-INT (P = 0.915). There were also no significant sex × time interactions for FCSA (P = 0.089), fibre type composition (P = 0.715), CD (P = 0.716), C:F (P = 0.216), Log_RSD (P = 0.150), SDH-OD (P = 0.678) and LCFR/SDH-INT (P = 0.355). Therefore, men and women were pooled for further analysis.

FCSA

FCSA was larger in the soleus than in the vastus lateralis muscle (4647 ± 1308 μm^2 *vs*. 3671 ± 1219 μm^2, $P < 0.001$). Irrespective of AG, the FCSA in both vastus lateralis and soleus muscles showed a progressive decline with bed rest, as reflected by the smaller FCSA at HDT6 than at BDC ($P < 0.001$, Hedge's g = 0.955) and a further decline in FCSA at HDT55 ($P < 0.001$, Hedge's g = 0.572) (Figure 4A). This decrease was similar for all fibre types, as reflected by the absence of a fibre type × time interaction. The FCSA was reduced more (−23.2 ± 12.4%) between BDC and HDT6 compared with the decline between HDT6 and HDT55 (−11.6 ± 12.1%) (P = 0.012). The standard deviation of FCSA did not change with bed rest (P = 0.127, data not shown).

Fibre type composition

The muscle × fibre type interaction for fibre type composition ($P < 0.001$) was reflected by a higher proportion of Type I fibres in the soleus than in the vastus lateralis muscle (all $P < 0.001$). Both the vastus lateralis and soleus muscles contained more Types I and IIa than Type IIx fibres ($P \leq 0.005$) and the soleus contained more Type I than Type IIa fibres ($P < 0.001$). Bed rest did not induce a significant change in fibre type composition (Table 1) (P = 0.972).

Muscle capillarization

Capillary density was higher at HDT55 than BDC (P = 0.002, Hedge's g = 0.703), irrespective of AG (P = 0.182) (*Figure* 3A). C:F, however, was reduced below BDC at HDT6 ($P < 0.001$ Hedge's g = 0.826) with no further decrease between HDT6 and HDT55 (P = 0.459) (*Figure* 3B). The Log_RSD was increased after 55 days of bed rest (P = 0.004, Hedge's g = 0.850) (*Figure* 3C).

The LCFR was greater in the soleus compared with the vastus lateralis muscle ($P < 0.001$), but there was no significant difference in CFD between soleus and vastus lateralis muscles (P = 0.217) (Table 2). Type I and IIa fibres had a greater LCFR compared with Type IIx fibres (both $P < 0.001$; Table 2), and Type I fibres had greater CFD than Type IIa and IIx fibres (P = 0.003 and P = 0.010, respectively).

There was a time effect for LCFR (P = 0.046), and post hoc analysis showed a significant decrease after 55 days (P = 0.043, Hedge's g = 0.270). There was also a time effect for CFD ($P < 0.001$), and post hoc analysis showed that CFD was greater in HDT55 relative to BDC (P = 0.001, Hedge's g = 0.389) (*Table* 2).

SDH

In all fibres pooled, there was no significant difference in SDH-OD between the vastus lateralis and soleus muscles (P = 0.141), although SDH-INT was greater in the soleus muscle (P = 0.001) (*Figure* 4B). Type I fibres had a higher SDH-OD than Type IIa, and Type IIa in turn had a greater SDH-OD than Type IIx fibres ($P < 0.001$; Table 3). The SDH-INT did not differ significantly between groups, or between muscle fibre types (*Table* 3 and *Figure* 4C). Both the SDH-OD (*Figure* 4B) and SDH-INT (*Figure* 4C) were not significantly different

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Table 1 Fibre cross-sectional area (FCSA) and fibre type composition in the control and the artificial gravity (AG) group 1 day prior to bed rest (BDC) and on Day 6 (HDT6) and Day 55 or 57 (HDT55/57) of head-down tilt bed rest

		BDC	HDT6	HDT55/57	Significant effects ($P < 0.05$)
			VL FCSA (μm^2)		
Control	Type I	4513 ± 756 (n = 6)	3410 ± 511 (n = 6)	2417 ± 280 (n = 6)	SOL > VL ($P < 0.001$)
AG	Type I	4269 ± 1286 (n = 13)	3581 ± 1052 (n = 13)	2906 ± 836 (n = 10)	BDC > 6 ($P < 0.001$)
Control	Type IIa	4980 ± 1071	3635 ± 730	2540 ± 536	BDC > 55 ($P < 0.001$)
AG	Type IIa	4390 ± 1259	3699 ± 1201	3208 ± 1044	6 > 55 ($P < 0.001$)
Control	Type IIx	4707 ± 1286	3386 ± 997	2506 ± 527	
AG	Type IIx	3797 ± 1369	3034 ± 823	2832 ± 1163	
			Fibre type composition (%)		
Control	Type I	37.7 ± 14.7	42.3 ± 13.8	39.2 ± 12.0	I > IIx ($P = 0.005$)
AG	Type I	35.8 ± 15.6	29.7 ± 9.6	34.3 ± 9.6	IIa > IIx ($P < 0.002$)
Control	Type IIa	33.2 ± 15.8	34.3 ± 7.8	34.6 ± 7.2	
AG	Type IIa	36.7 ± 9.7	41.1 ± 11.3	37.7 ± 8.0	
Control	Type IIx	29.1 ± 12.1	22.4 ± 11.1	25.8 ± 6.1	
AG	Type IIx	27.4 ± 14.1	28.8 ± 11.4	28.1 ± 13.3	
			SOL FCSA (μm^2)		
Control	Type I	5241 ± 1011 (n = 6)	4494 ± 718 (n = 6)	3585 ± 826 (n = 5)	SOL > VL ($P < 0.001$)
AG	Type I	5397 ± 1346 (n = 12)	4390 ± 1169 (n = 12)	3767 ± 1355 (n = 12)	BDC > 6 ($P < 0.001$)
Control	Type IIa	6211 ± 1650	4784 ± 1028	4275 ± 1213	BDC > 55 ($P < 0.001$)
AG	Type IIa	5859 ± 1277	4144 ± 1238	3794 ± 1188	6 > 55 ($P < 0.001$)
Control	Type IIx		3416 ± 607	4497 ± 2321	
AG	Type IIx	5545 ± 1510	4054 ± 1478	3507 ± 1349	
			Fibre type composition (%)		
Control	Type I	71.0 ± 18.8	62.7 ± 21.6	62.4 ± 19.6	I > IIa ($P < 0.001$)
AG	Type I	60.2 ± 23.6	63.6 ± 17.6	59.7 ± 18.1	I > IIx ($P < 0.001$)
Control	Type IIa	27.2 ± 17.6	32.3 ± 17.4	29.4 ± 8.9	IIa > IIx ($P < 0.001$)
AG	Type IIa	32.4 ± 17.2	27.8 ± 8.6	29.5 ± 9.1	
Control	Type IIx	1.7 ± 4.2	5.0 ± 9.2	8.2 ± 12.7	
AG	Type IIx	7.5 ± 12.7	8.6 ± 14.8	10.9 ± 13.2	

SOL, *soleus muscle*; VL, *vastus lateralis muscle*.
Data are mean ± standard deviation.

from BDC at HDT6 (P = 0.120 and P = 0.522, respectively), but was decreased at HDT55 relative to BDC ($P < 0.001$, Hedge's g = 0.801).

Supply–demand ratio

To determine differences in the matching of oxygen supply (LCFR) to demand (SDH-INT) of a fibre, the LCFR/SDH-INT was calculated. The LCFR/SDH-INT was similar in BDC and HDT6 (P = 0.522) but was elevated at HDT55 ($P < 0.001$, Hedge's g = 0.956; *Figure* 4D).

PO_2 modelling

The average PO_2 at maximal oxygen consumption of the fibres calculated with the oxygen transport modeller programme did not differ significantly between BDC and HDT6 (P = 1.000), but was greater at HDT55 relative to BDC (P = 0.010, Hedge's g = 0.726) and HDT6 (P = 0.010, Hedge's g = 0.811) (*Figure* 3D).

Discussion

The main observations of this study were that fibre atrophy and capillary rarefaction, illustrated by reductions in FCSA and C:F, respectively, seen after just 6 days of bed rest are proportional. The atrophy proceeded at a slower rate between 6 and 55 days of bed rest and was accompanied by a reduction in oxidative capacity, without any further capillary rarefaction. As a result, after 55 days of bed rest, the capillary supply is in excess to that expected from the oxidative capacity and size of the muscle fibres. Daily artificial gravity failed to blunt the bed-rest-induced reductions in fibre size, oxidative capacity and capillary rarefaction.

Fibre size and fibre type composition

We observed no significant changes in fibre type composition in either muscle at any time point, similar to findings after 19 days of bed rest.[22] Others, however, did report a reduced proportion of slow fibres after 60 days of bed rest in the

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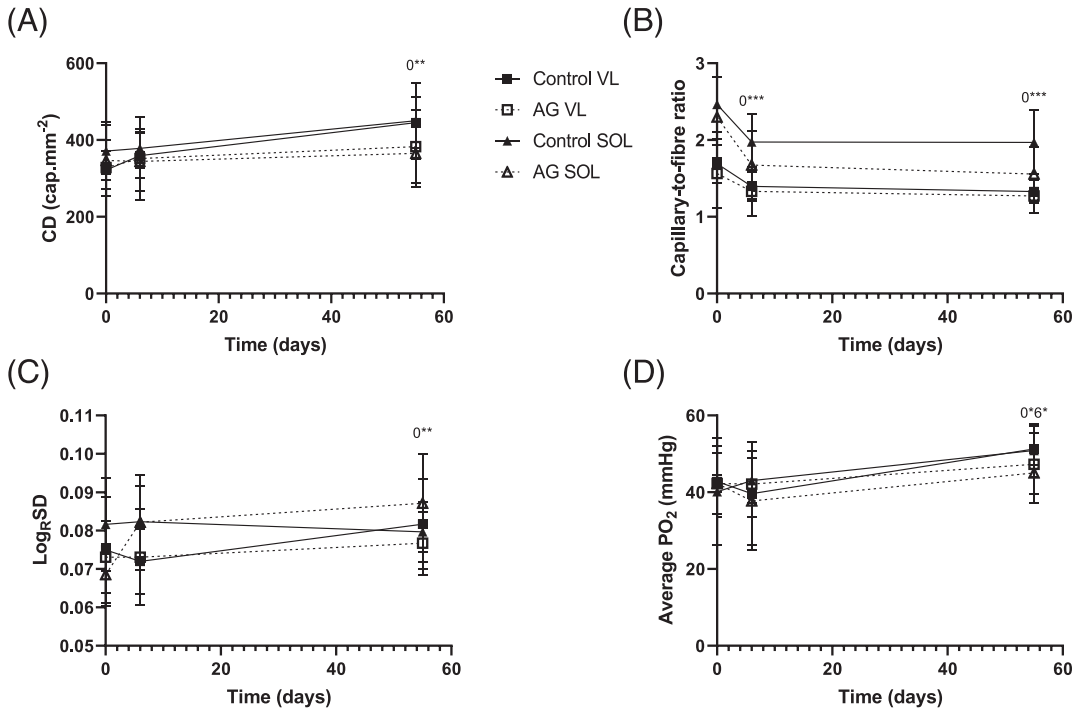

Figure 3 Capillary indices for control group and antigravity (AG) group at baseline, on Day 6 of head down tilt bed rest and on Day 55 of head-down tilt bed rest ± standard deviation. (A) Capillary density (CD), (B) capillary-to-fibre ratio (C:F), (C) the logarithmic standard deviation of the radius of the capillary domains (Log_RSD) (D) Average PO_2. SOL, soleus; VL, vastus lateralis, 0* significantly different to baseline at $P < 0.05$, 0** significantly different to baseline at $P < 0.01$, 0*** significantly different to baseline at $P < 0.001$, 6* significantly different to Day 6 at $P < 0.05$.

soleus, but not in the vastus lateralis muscle.[23] Although the discrepancy between that and our study may be explicable by the classification of hybrid fibres in their study, this will have a minor impact on the outcome as they reported no significant change in the proportion of hybrid fibres after 60 days of bed rest.[23]

Reductions in muscle volume with space flight are well documented[24,25] and are largely attributable to muscle fibre atrophy.[23,26] In line with other studies showing significant muscle atrophy within 1 week of bed rest,[3,4,27] we observed notable fibre atrophy in both vastus lateralis and soleus muscles after just 6 days. Here, we confirm that the rate of atrophy is particularly fast during this first week as the further atrophy between 6 and 55 days of bed rest was just around 50% of that incurred during the first week. It may well be that muscle fibre atrophy reaches a plateau, as seen in denervated rodent muscles where after rapid atrophy in the first 2 weeks, muscle mass did not decrease further, reaching a new steady state.[28] That this may indeed also be the case in humans is supported by a recent systematic review, which demonstrated that muscle atrophy follows a non-linear logarithmic pattern until 3 months.[5] It would be of interest to understand the molecular mechanisms underlying the time course of bed-rest-induced atrophy, but this was beyond the scope of the present study.

Such a rapid loss of muscle mass within just 6 days of immobilization is not only an important consideration for spaceflight but has also significant implications for patients when one considers that the average length of hospital stay for in-patients in the UK is 6.8 days.[29] Given that low skeletal muscle cross-sectional area is a risk factor for mortality in critically ill patients,[30] the observation of a particularly rapid atrophy during the first week of bed rest demonstrates the importance of early interventions to attenuate reductions in muscle size.

Muscle oxidative capacity

In contrast to the early development of atrophy, the oxidative capacity of muscle fibres was not reduced after 6 days of bed rest, and only after 55 days of bed rest, a significant reduction was seen in both muscles and in all fibre types. Similar reductions have been found in both the vastus lateralis and soleus after 19[22] and 37[31] days of bed rest. The most straightforward explanation for this observation is that after extended bed rest, the loss of mitochondria is proportionately larger than the decrease in fibre size. These histological observations are in line with our preliminary findings of a significant reduction in the maximum mitochondrial respiration rate of

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Table 2 Local capillary-to-fibre ratio (LCFR) and capillary fibre density (CFD) of each fibre type for the control and the artificial gravity (AG) group 1 day prior to bed rest (BDC) and on Day 6 (HDT6) and Day 55 or 57 (HDT55/57) of head-down tilt bed

		BDC	HDT6	HDT55/57	Significant effects ($P < 0.05$)
			VL LCFR		
Control	Type I	1.44 ± 0.32	1.34 ± 0.39	1.11 ± 0.15	SOL > VL ($P < 0.001$)
AG	Type I	1.30 ± 0.44	1.33 ± 0.33	1.22 ± 0.35	I > IIx ($P < 0.001$)
Control	Type IIa	1.51 ± 0.20	1.37 ± 0.39	1.19 ± 0.26	IIa > IIx ($P < 0.001$)
AG	Type IIa	1.20 ± 0.34	1.21 ± 0.34	1.26 ± 0.37	BDC > 55 ($P = 0.043$)
Control	Type IIx	1.31 ± 0.34	1.12 ± 0.36	0.99 ± 0.22	
AG	Type IIx	0.93 ± 0.26	0.93 ± 0.27	0.86 ± 0.21	
CFD (mm^{-2})					
Control	Type I	309 ± 41	332 ± 88	403 ± 49	Time ($P < 0.001$)
AG	Type I	312 ± 69	319 ± 77	336 ± 97	HDT55 > BDC ($P = 0.001$)
Control	Type IIa	298 ± 46	300 ± 87	368 ± 69	I > IIa ($P = 0.003$)
AG	Type IIa	295 ± 63	322 ± 51	387 ± 83	I > IIx ($P = 0.010$)
Control	Type IIx	274 ± 49	301 ± 87	297 ± 55	BDC < HDT55 ($P = 0.001$)
AG	Type IIx	282 ± 94	317 ± 113	327 ± 114	
			SOL LCFR		
Control	Type I	1.83 ± 0.26	1.79 ± 0.29	1.70 ± 0.50	SOL > VL ($P < 0.001$)
AG	Type I	1.84 ± 0.61	1.50 ± 0.45	1.35 ± 0.48	I > IIx ($P < 0.001$)
Control	Type IIa	1.76 ± 0.37	1.70 ± 0.35	1.90 ± 0.50	IIa > IIx ($P < 0.001$)
AG	Type IIa	1.76 ± 0.51	1.47 ± 0.42	1.28 ± 0.24	BDC > 55 ($P = 0.043$)
Control	Type IIx	2.05 ± 0.73	1.31 ± 0.44	1.52 ± 0.56	
AG	Type IIx	1.45 ± 0.65	1.16 ± 0.27	1.10 ± 0.52	
			CFD (mm^{-2})		
Control	Type I	344 ± 84	357 ± 73	425 ± 122	HDT55 > BDC ($P = 0.001$)
AG	Type I	367 ± 84	351 ± 84	357 ± 87	I > IIa ($P = 0.003$)
Control	Type IIa	295 ± 63	322 ± 51	387 ± 83	I > IIx ($P < 0.010$)
AG	Type IIa	340 ± 68	341 ± 88	331 ± 73	BDC < HDT55 ($P = 0.001$)
Control	Type IIx	261 ± 23	314 ± 28	323 ± 75	
AG	Type IIx	328 ± 101	337 ± 142	309 ± 66	

SOL, *soleus muscle*; VL, *vastus lateralis muscle*.
Data are mean ± standard deviation.

permeabilized fibres of the vastus lateralis muscle after 55 days of bed rest.[32] Although mitochondrial dysfunction after 55 days of bed rest may explain such a decrease in muscle oxidative capacity, it has been shown that after 10 days of non-head down bed rest, the protein expression of subunits of the mitochondrial complexes and citrate synthase is reduced without evidence of a lower maximal mitochondrial respiration rate.[4,33] This indicates no, or a very small, reduction in oxidative phosphorylation capacity, further supported by observations such as an absence of oxidative stress in the muscle or increases in ROS production after 4–7 days of bed rest.[34,35] In fact, there is evidence that skeletal muscle mitochondrial respiration is even augmented after 4 days of bed rest.[34]

Capillarization and capillary supply-to-oxygen demand ratio

During periods of bed rest, there are few instances of contractile activity and associated increases in muscle blood flow. As a result, the endothelium is exposed to less mechanical stress due to muscle contraction and/or blood-flow-related increases in sheer stress, factors that are important for angiogenesis and maintenance of the capillary bed.[12] The reduced contractile activity and endothelial shear stress may explain the microvascular rarefaction, demonstrated by a decreased C:F in both the soleus and vastus lateralis muscle, after as little as 6 days of bed rest. Yet, the CD was not significantly changed. Whereas capillary rarefaction during bed rest is not always observed,[4,22] those same studies did not demonstrate significant fibre atrophy and, similar to what we saw in our study, no change in CD. Although one study observed atrophy without capillary rarefaction and an increase in CD after 4 days of bed rest,[3] the pattern emerges that the loss of capillaries—when it occurs—is proportional to the decrease in fibre size—as reflected by the absence of a decrease in CD—confirming the coupling between the size and the capillary supply of a fibre[13] even during early stages of bed rest.

Other studies observing a significant reduction in C:F in the vastus lateralis muscle from middle-aged participants after 14[36] or 60 days of bed rest[23] may suggest that the loss of capillaries is progressive. However, we have shown here that

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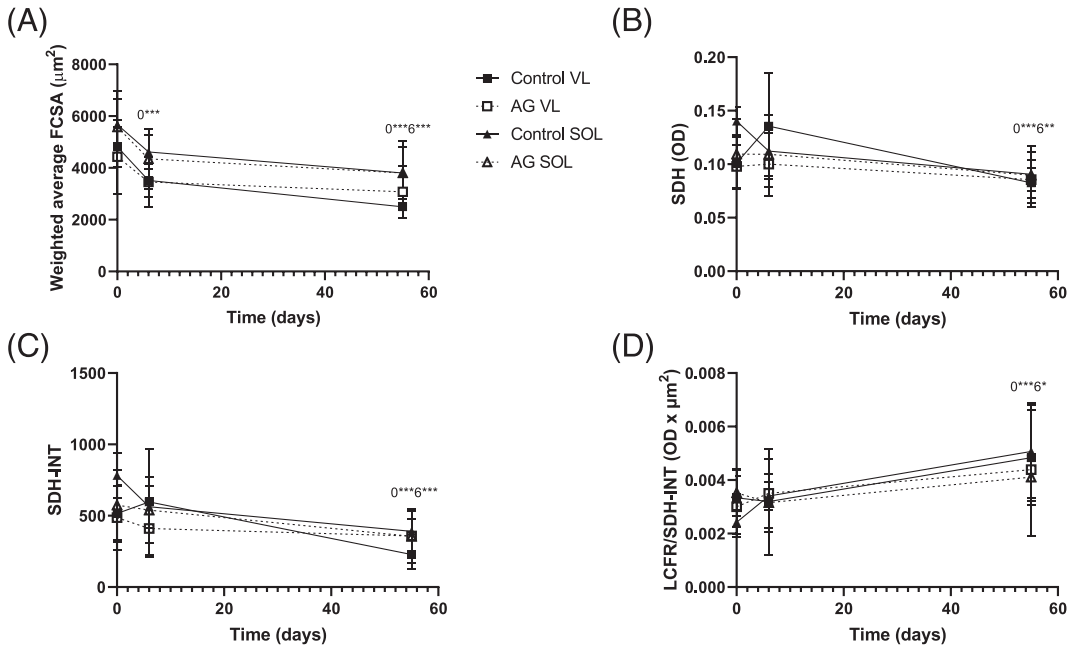

Figure 4 (A) Weighted average fibre cross-sectional area (FCSA) for control group and antigravity (AG) group at baseline, on Day 6 of head down tilt bed rest and on Day 55 of head-down tilt bed rest ± standard deviation. (B) shows average succinate dehydrogenase activity (SDH-OD). (C) Integrated SDH (SDH-INT). (D) Local capillary-to-fibre ratio LCFR: SDH-INT. LCFR, local capillary-to-fiber ratio; SOL, soleus; VL, vastus lateralis. 0* significantly different to baseline at $P < 0.05$, 0*** significantly different to baseline at $P < 0.001$, 1*** significantly different to baseline at $P < 0.001$, 6** significantly different to Day 6 at $P < 0.01$, 6*** significantly different to Day 6 of bed rest at $P < 0.001$.

almost all, if not all, capillary rarefaction occurs during the first 6 days without any further significant capillary rarefaction between 6 and 55 days of bed rest despite further muscle fibre atrophy. As a result, the CD was higher at 55 days of bed rest than before bed rest, and this indicates that at later stages of disuse, there may be an uncoupling of fibre size and capillary supply to a muscle fibre, something also seen in denervated rodent soleus muscles.[28]

One thing that is rarely considered is the spatial distribution of capillaries over the muscle tissue. Indeed, model calculations have shown that the heterogeneity of capillary spacing has a significant impact on muscle oxygenation.[37] We therefore quantified here also the heterogeneity of capillary spacing and showed that despite significant capillary rarefaction with bed rest, the spatial distribution was not significantly changed after 6 days. This indicates that the capillary

Table 3 Succinate dehydrogenase optical density (SDH-OD) of each fibre type for the control and artificial gravity (AG) group 1 day prior to bed rest (BDC) and on Day 6 (HDT6) and Day 55 or 57 (HDT55/57) of head-down tilt bed rest

		BDC	HDT6	HDT55/57	Significant effects ($p < 0.05$)
			SDH-OD VL		
Control	Type I	0.124 ± 0.024	0.144 ± 0.050	0.115 ± 0.033	I > IIa ($P < 0.001$)
AG	Type I	0.113 ± 0.014	0.123 ± 0.033	0.105 ± 0.030	I > IIx ($P < 0.001$)
Control	Type IIa	0.098 ± 0.026	0.132 ± 0.049	0.084 ± 0.019	IIa > IIx ($P < 0.001$)
AG	Type IIa	0.095 ± 0.023	0.093 ± 0.034	0.072 ± 0.022	BDC > 55 ($P < 0.001$)
Control	Type IIx	0.076 ± 0.029	0.109 ± 0.056	0.071 ± 0.017	6 > 55 ($P = 0.006$)
AG	Type IIx	0.078 ± 0.028	0.075 ± 0.022	0.063 ± 0.019	
			SOL		
Control	Type I	0.147 ± 0.009	0.121 ± 0.031	0.099 ± 0.023	I > IIa ($P < 0.001$)
AG	Type I	0.118 ± 0.030	0.117 ± 0.021	0.097 ± 0.019	I > IIx ($P < 0.001$)
Control	Type IIa	0.127 ± 0.027	0.096 ± 0.039	0.080 ± 0.026	IIa > IIx ($P < 0.001$)
AG	Type IIa	0.101 ± 0.030	0.099 ± 0.023	0.076 ± 0.021	BDC > 55 ($P < 0.001$)
Control	Type IIx	0.138 ± 0.044	0.066 ± 0.009	0.071 ± 0.033	6 > 55 ($P = 0.006$)
AG	Type IIx	0.091 ± 0.038	0.077 ± 0.020	0.068 ± 0.016	

SOL, *soleus muscle*; VL, *vastus lateralis muscle*.
Data are mean ± standard deviation.

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rarefaction after 6 days of bed rest does not occur at random, but is apparently a controlled process to preserve adequate muscle oxygenation, and removal of heat and waste products. However, after 55 days, even though C:F is maintained, the heterogeneity of capillary spacing is increased. This increase in heterogeneity of capillary spacing may be related to changes in fibre size as reflected also by the increase in CD, but is not attributable to an increased variability of the FCSA.

Above we have discussed that after 55 days of bed rest, the loss of oxidative capacity is proportionally larger than the decrease in fibre size, which in turn is proportionally larger than the capillary rarefaction. The combination of these changes results in a muscle fibre capillarization that is larger than expected for a given fibre size and oxidative capacity, or, in other words, a capillary supply that exceeds the oxygen demand and thus resulting in a better, rather than poorer muscle oxygenation in advanced stages of bed rest. This mismatch between oxygen supply and demand has also been found after 19 days of bed rest, where the decrement in muscle fibre oxidative capacity was proportionally greater than the loss of capillaries[22] and in old rat muscles without muscle atrophy[38] and the soleus[28] muscles atrophied due to denervation. These observations provide further evidence that capillarization is not determined by oxidative capacity but by other factors, possibly including substrate delivery or metabolite removal.[12,13] It appears that there is not only an uncoupling of the link between the size and capillary supply of a fibre during bed rest but that also capillary rarefaction (after 6 days of bed rest) and loss of oxidative capacity follow a different time course.

Figure 5 illustrates that during the first days of bed rest the relationship between FCSA and C:F is maintained (A to B), followed by a further decrease in fibre size without significant capillary rarefaction (B to C), resulting in an elevated capillary supply for a given fibre size. Thus, although the initial capillary rarefaction may have contributed to the decrease in fibre size, the uncoupled capillarization after 55 days of bed rest may put the muscle in an advantageous position for subsequent recovery. In support of this suggestion, it has been shown that a denser microvascular network enhances the hypertrophic responses to resistance training in older people.[15] *Figure* 5 illustrates that this higher capillary density allows fibre growth without the requirement of capillary proliferation, which is significant as angiogenesis may well be impaired after a period of immobilization.[39]

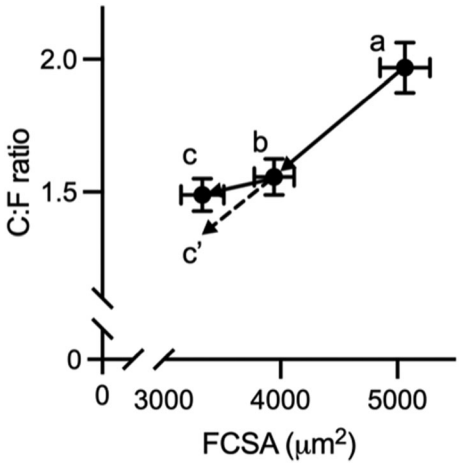

Figure 5 The relationship between fibre cross-sectional area (FCSA) and capillary-to-fibre ratio (C:F) at BDC (A), HDT6 (B) and HDT55/57 (C). C' indicates the C:F expected for the FCSA at (C). Data are mean ± SEM.

Effects of artificial gravity

Contrary to our expectation, the artificial gravity intervention did not attenuate the bed-rest-induced decrements in fibre size, capillarization or oxidative capacity. Other studies, however, have found benefits of artificial gravity. The discrepancy may be related to the duration and/or applied G-forces, where in our study participants underwent 30 min of artificial gravity of 2G at the foot, compared with 1 h and 2.5 G in the other studies.[17,18] Therefore, it may be that the dose and duration of artificial gravity intervention were insufficient to induce the muscle-sparing effect. Even though artificial gravity did not attenuate the bed-rest-induced muscle atrophy, loss of oxidative capacity and capillary rarefaction, the absence of any negative effects of centrifugation in the present study supports the use of this modality if it is effective in mitigating other unfavourable effects of bed rest.

Limitations

Further preliminary confirmation of the decrease in oxidative capacity with bed rest has been provided through the direct measurement of oxidative phosphorylation with permeabilized muscle fibres.[32] However, the method of quantitative histochemistry of SDH used in the present study allowed us to study the relationship between oxygen supply and oxidative demand of muscle fibres within the context of the capillary bed in muscle cross sections in the form of LCFR/SDH-INT and modelled estimates of PO_2, something that cannot be done in saponin-treated fibres.

Conclusions

Significant muscle fibre atrophy and capillary rarefaction, as reflected by the decreased C:F ratio, can be observed after just 6 days of bed rest in both the postural soleus and non-postural vastus lateralis muscle. This indicates that early interventions may be necessary to prevent these decrements in muscle morphology. In addition, the relationship between fibre size and oxidative capacity with the capillary supply of

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a fibre is uncoupled during prolonged bed rest as reflected by a rapid loss of muscle mass and capillaries, followed at later stages by a more than proportional loss of mitochondria without further capillary loss. The resulting excessive capillary supply of the muscle after prolonged bed rest is advantageous for the delivery of substrates needed for subsequent muscle recovery.

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Ethics statement

This study was approved by the Ethics Committee of the North Rhine Medical Association (reference number 2018143) in Düsseldorf, Germany, and was included in the German Clinical Trials Register (DRKS-ID: DRKS00015677). All participants gave written informed consent prior to the study. The study adhered to the standards of the Declaration of Helsinki and complied with the ethical guidelines for authorship and publishing in the *Journal of Cachexia, Sarcopenia and Muscle*.[40]

Conflict of interest

The authors declare that they have no conflicts of interest.

References

1. Wall BT, Dirks ML, Snijders T, van Dijk JW, Fritsch M, Verdijk LB, et al. Short-term muscle disuse lowers myofibrillar protein synthesis rates and induces anabolic resistance to protein ingestion. *Am J Physiol Endocrinol Metab* 2016;**310**: E137–E147.
2. Belavý DL, Miokovic T, Armbrecht G, Richardson CA, Rittweger J, Felsenberg D. Differential atrophy of the lower-limb musculature during prolonged bed-rest. *Eur J Appl Physiol* 2009;**107**:489–499.
3. Montero D, Oberholzer L, Haider T, Breenfeldt-Andersen A, Dandanell S, Meinild-Lundby AK, et al. Increased capillary density in skeletal muscle is not associated with impaired insulin sensitivity induced by bed rest in healthy young men. *Can J Appl Physiol* 2018;**43**: 1334–1340.
4. Dirks ML, Wall BT, van de Valk B, Holloway TM, Holloway GP, Chabowski A, et al. One week of bed rest leads to substantial muscle atrophy and induces whole-body insulin resistance in the absence of skeletal muscle lipid accumulation. *Diabetes* 2016; **65**:2862–2875.
5. Marusic U, Narici M, Simunic B, Pisot R, Ritzmann R. Nonuniform loss of muscle strength and atrophy during bed rest: A systematic review. *J Appl Physiol (1985)* 2021;**131**:194–206.
6. Debevec T, Ganse B, Mittag U, Eiken O, Mekjavic IB, Rittweger J. Hypoxia aggravates inactivity-related muscle wasting. *Front Physiol* 2018;**9**:494.
7. Kramer A, Gollhofer A, Armbrecht G, Felsenberg D, Gruber M. How to prevent the detrimental effects of two months of bed-rest on muscle, bone and cardiovascular system: An RCT. *Sci Rep* 2017;**7**:13177.
8. Degens H, Veerkamp JH. Changes in oxidative capacity and fatigue resistance in skeletal muscle. *Int J Biochem* 1994;**26**: 871–878.
9. Weber T, Ducos M, Mulder E, Beijer Å, Herrera F, Zange J, et al. The relationship between exercise-induced muscle fatigue, arterial blood flow and muscle perfusion after 56 days local muscle unloading. *Clin Physiol Funct Imaging* 2014;**34**:218–229.
10. Bosutti A, Mulder E, Zange J, Bühlmeier J, Ganse B, Degens H. Effects of 21 days of bed rest and whey protein supplementation on plantar flexor muscle fatigue resistance during repeated shortening contractions. *Eur J Appl Physiol* 2020;**120**: 969–983.
11. Tickle PG, Hendrickse PW, Degens H, Egginton S. Impaired skeletal muscle performance as a consequence of random functional capillary rarefaction can be restored with overload-dependent angiogenesis. *J Physiol* 2020;**598**:1187–1203.
12. Hendrickse P, Degens H. The role of the microcirculation in muscle function and plasticity. *J Muscle Res Cell Motil* 2019;**40**: 127–140.
13. Bosutti A, Egginton S, Barnouin Y, Ganse B, Rittweger J, Degens H. Local capillary supply in muscle is not determined by local oxidative capacity. *J Exp Biol* 2015;**218**: 3377–3380.
14. Plyley MJ, Olmstead BJ, Noble EG. Time course of changes in capillarization in hypertrophied rat plantaris muscle. *J Appl Physiol (1985)* 1998;**84**:902–907.
15. Snijders T, Nederveen JP, Joanisse S, Leenders M, Verdijk LB, van Loon LJ, et al. Muscle fibre capillarization is a critical factor in muscle fibre hypertrophy during resistance exercise training in older men. *J Cachexia Sarcopenia Muscle* 2017;**8**: 267–276.
16. Thijssen DH, Green DJ, Hopman MT. Blood vessel remodeling and physical inactivity in humans. *J Appl Physiol (1985)* 2011;**111**: 1836–1845.
17. Caiozzo VJ, Haddad F, Lee S, Baker M, Paloski W, Baldwin KM. Artificial gravity as a countermeasure to microgravity: A pilot study examining the effects on knee extensor and plantar flexor muscle groups. *J Appl Physiol (1985)* 2009;**107**:39–46.
18. Frett T, Green DA, Mulder E, Noppe A, Arz M, Pustowalow W, et al. Tolerability

of daily intermittent or continuous short-arm centrifugation during 60-day 6o head down bed rest (AGBRESA study). *PLoS ONE* 2020;**15**:e0239228.
19. van der Laarse WJ, Diegenbach PC, Elzinga G. Maximum rate of oxygen consumption and quantitative histochemistry of succinate dehydrogenase in single muscle fibres of *Xenopus laevis*. *J Muscle Res Cell Motil* 1989;**10**:221–228.
20. al-Shammari AA, Kissane RWP, Holbek S, Mackey AL, Andersen TR, Gaffney EA, et al. Integrated method for quantitative morphometry and oxygen transport modeling in striated muscle. *J Appl Physiol (1985)* 2019;**126**:544–557.
21. Barnouin Y, McPhee JS, Butler-Browne G, Bosutti A, de Vito G, Jones DA, et al. Coupling between skeletal muscle fiber size and capillarization is maintained during healthy aging. *J Cachexia Sarcopenia Muscle* 2017;**8**:647–659.
22. Bosutti A, Salanova M, Blottner D, Buehlmeier J, Mulder E, Rittweger J, et al. Whey protein with potassium bicarbonate supplement attenuates the reduction in muscle oxidative capacity during 19 days of bed rest. *J Appl Physiol (1985)* 2016;**121**:838–848.
23. Blottner D, Hastermann M, Weber R, Lenz R, Gambara G, Limper U, et al. Reactive jumps preserve skeletal muscle structure, phenotype, and myofiber oxidative capacity in bed rest. *Front Physiol* 2020;**10**:1527.
24. Edgerton VR, Zhou MY, Ohira Y, Klitgaard H, Jiang B, Bell G, et al. Human fiber size and enzymatic properties after 5 and 11 days of spaceflight. *J Appl Physiol (1985)* 1995;**78**:1733–1739.
25. Akima H, Kawakami Y, Kubo K, Sekiguchi C, Ohshima H, Miyamoto A, et al. Effect of short-duration spaceflight on thigh and leg muscle volume. *Med Sci Sports Exerc* 2000;**32**:1743–1747.
26. Thot GK, Berwanger C, Mulder E, Lee JK, Lichterfeld Y, Ganse B, et al. Effects of long-term immobilisation on endomysium of the soleus muscle in humans. *Exp Physiol* 2021;**106**:2038–2045.
27. Mulder E, Clément G, Linnarsson D, Paloski WH, Wuyts FP, Zange J, et al. Musculoskeletal effects of 5 days of bed rest with and without locomotion replacement training. *Eur J Appl Physiol* 2015;**115**:727–738.
28. Degens H, Kosar SN, Hopman MT, de Haan A. The time course of denervation-induced changes is similar in soleus muscles of adult and old rats. *Appl Physiol Nutr Metab* 2008;**33**:299–308.
29. Hospital discharges and length of stay statistics. Statistics Explained. 2021. https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Hospital_discharges_and_length_of_stay_statistics#Average_length_of_hospital_stay_for_in-patients
30. Weijs PJ, Looijaard WG, Dekker IM, Stapel SN, Girbes AR, Straaten H, et al. Low skeletal muscle area is a risk factor for mortality in mechanically ventilated critically ill patients. *Crit Care* 2014;**18**:R12.
31. Ferretti G, Antonutto G, Denis C, Hoppeler H, Minetti AE, Narici MV, et al. The interplay of central and peripheral factors in limiting maximal O2 consumption in man after prolonged bed rest. *J Physiol* 1997;**501**:677–686.
32. Giakoumaki I, Ganse B, Rittweger J, Degens H, Wüst RCI. *Mitochondrial respiration in human skeletal muscle after 60 days of bed rest*. Texas, USA: Galveston Island; 2020.
33. Zuccarelli L, Baldassarre G, Magnesa B, Degano C, Comelli M, Gasparini M, et al. Peripheral impairments of oxidative metabolism after a 10-day bed rest are upstream of mitochondrial respiration. *J Physiol* 2021;**599**:4813–4829.
34. Larsen S, Lundby AM, Dandanell S, Oberholzer L, Keiser S, Andersen AB, et al. Four days of bed rest increases intrinsic mitochondrial respiratory capacity in young healthy males. *Physiol Rep* 2018;**6**:e13793.
35. Dirks ML, Miotto PM, Goossens GH, Senden JM, Petrick HL, van Kranenburg J, et al. Short-term bed rest-induced insulin resistance cannot be explained by increased mitochondrial H2O2 emission. *J Physiol* 2020;**598**:123–137.
36. Arentson-Lantz EJ, English KL, Paddon-Jones D, Fry CS. Fourteen days of bed rest induces a decline in satellite cell content and robust atrophy of skeletal muscle fibers in middle-aged adults. *J Appl Physiol (1985)* 2016;**120**:965–975.
37. Degens H, Deveci D, Botto-van Bemden A, Hoofd LJ, Egginton S. Maintenance of heterogeneity of capillary spacing is essential for adequate oxygenation in the soleus muscle of the growing rat. *Microcirculation* 2006;**13**:467–476.
38. Hepple RT, Vogell JE. Anatomic capillarization is maintained in relative excess of fiber oxidative capacity in some skeletal muscles of late middle-aged rats. *J Appl Physiol (1985)* 2004;**96**:2257–2264.
39. Gliemann L, Rytter N, Jørgensen TS, Piil P, Carter H, Nyberg M, et al. The Impact of Lower Limb Immobilization and Rehabilitation on Angiogenic Proteins and Capillarization in Skeletal Muscle. *Med Sci Sports Exerc* 2021;**53**:1797–1806.
40. von Haehling S, Morley JE, Coats AJS, Anker SD. Ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle: Update 2021. *J Cachexia Sarcopenia Muscle* 2021;**12**:2259–2261.