Human single muscle fibre function with 84 day bed-rest and resistance exercise

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Muscle biopsies were obtained from the vastus lateralis before and after 84 days of bed-rest from six control (BR) and six resistance-exercised (BRE) men to examine slow- and fast-twitch muscle fibre contractile function. BR did not exercise during bed-rest and had a 17 and 40% decrease in whole muscle size and function, respectively. The BRE group performed four sets of seven maximal concentric and eccentric supine squats 2–3 days per week (every third day) that maintained whole muscle strength and size. Slow (MHC I) and fast (MHC IIa) muscle fibres were studied at 15°C for diameter, peak force (P_o), contractile velocity (V_o) and force–power parameters. SDS-PAGE was performed on each single fibre after the functional experiments to determine MHC isoform composition. MHC I and IIa BR fibres were, respectively, 15 and 8% smaller, 46 and 25% weaker (P_o), 21 and 6% slower (V_o), and 54 and 24% less powerful after bed-rest ($P < 0.05$). BR MHC I and IIa P_o and power normalized to cell size were lower ($P < 0.05$). BRE MHC I fibres showed no change in size or V_o after bed-rest; however, P_o was 19% lower ($P < 0.05$), resulting in 20 and 30% declines ($P < 0.05$) in normalized P_o and power, respectively. BRE MHC IIa fibres showed no change in size, P_o and power after bed-rest, while V_o was elevated 13% ($P < 0.05$). BRE MHC IIa normalized P_o and power were 10 and 15% lower ($P < 0.05$), respectively. MHC isoform composition shifted away from MHC I fibres, resulting in an increase ($P < 0.05$) in MHC I/IIa (BR and BRE) and MHC IIa/IIx (BR only) fibres. These data show that the contractile function of the MHC I fibres was more affected by bed-rest and less influenced by the resistance exercise protocol than the MHC IIa fibres. Considering the large differences in power of human MHC I and IIa muscle fibres (5- to 6-fold), the maintenance of whole muscle function with the resistance exercise programme is probably explained by (1) the maintenance of MHC IIa power and (2) the shift from slow to fast (MHC I → MHC I/IIa) in single fibre MHC isoform composition.

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Spaceflight is a unique environment that poses several physiological challenges to the human body. As the various space agencies around the world focus their attention on long duration stays aboard the International Space Station (ISS), implementation of effective exercise regimens will be essential for the health and well being of the crew members. In particular, maintenance of the musculoskeletal system will be necessary for successful long duration space travel (Baldwin *et al.* 1996; NASA, 2000). Previous studies have documented that skeletal muscle mass and strength are reduced with as little as 7 days of spaceflight (LeBlanc *et al.* 1995) and continue to decline with the length of exposure (cf. Adams *et al.* 2003). The reduction in muscle mass accounts for about two-thirds of the decrease in muscle strength. Thus, other physiological mechanisms, such as alterations in neural drive or intrinsic changes to the muscles fibres may be contributing to the deleterious changes in skeletal muscle function with unloading.

Data to support changes in the cross-bridge mechanics of human single muscle fibres have been shown with 17 days of spaceflight (Widrick *et al.* 1999), 17 days of bed-rest (Widrick *et al.* 1997), 37 days of bed-rest (Larsson *et al.* 1996) and 4 months of bed-rest (Yamashita-Goto *et al.* 2001). Taken together, these studies found that muscle fibres have a lower force per cross-sectional area and an elevated shortening velocity (V_o) following the

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unloading period. Thus, it appears that alterations in the contractile properties of human muscle do occur during periods of weightlessness and bed-rest. Furthermore, the changes observed between spaceflight and bed-rest closely mimicked each other, providing further evidence that bed-rest is a good ground-based analogue for studying changes in human muscle function (Widrick *et al.* 1999; Trappe *et al.* 2001*c*; Adams *et al.* 2003).

Resistance training is the most promising candidate for providing the proper stimulus to maintain muscle function while in space. However, the optimal exercise protocol has not been established (Baldwin *et al.* 1996; NASA, 2000; Adams *et al.* 2003). Given the time and energy requirements of crew members on board the ISS, the space agencies are interested in implementing effective countermeasure activities that minimize the exercise time of the crew members while at the same time maximizing the physiological benefits. Recently, our laboratory found that resistance training every third day during 21 days of unloading was sufficient to maintain muscle mass and strength (Schulze *et al.* 2002). Our study and others (Tesch *et al.* 1990; Dudley *et al.* 1991; Bamman *et al.* 1998) have concluded that to maximize resistance-training benefits, a programme must include concentric and eccentric muscle actions at a high intensity. While these initial studies are promising they are limited by the short duration of unloading. It is unknown if the same type of positive results would carry over to longer periods of unloading such as the current duration of ISS missions (range ~90–180 days).

The intent of this investigation was to examine the alterations in the cellular mechanisms at the level of the single muscle fibre with long-duration (84 days) unloading in apparently healthy adults. In addition, high-intensity resistance training was employed in a separate group of volunteers to examine whether myocellular function could be maintained during a long-term period of unloading. We hypothesized that fibre diameter, force, force per cross-sectional area, power and normalized power would be reduced while shortening velocity would be elevated after bed-rest in subjects who did not perform any countermeasure activities. Conversely, we hypothesized that high-intensity resistance training every 2–3 days would be sufficient to maintain slow- and fast-twitch single fibre contractile characteristics with long-term bed-rest.

The unique aspects of this project were (1) the duration of bed-rest (84 days), (2) the use of a strict bed-rest (no countermeasures) control group for baseline measurements of muscle loss, (3) employment of resistance training every 2–3 days using a novel flywheel ergometer that has been designed for use in space (Berg & Tesch, 1994, 1998; Alkner *et al.* 2003; Tesch *et al.* 2003; Alkner & Tesch, 2004), and (4) measurement of contractile properties in individual slow- and fast-twitch muscle fibres from the vastus lateralis muscle.

Methods

Subjects

Twelve healthy men performed 90 days of 6 deg head-down tilt bed-rest. Subjects were divided into a bed-rest only (BR; $n = 6$) group and bed-rest with exercise (BRE; $n = 6$) group. Subjects in the BR group were 32 ± 2 years, 174 ± 2 cm, 70 ± 2 kg and had a BMI of 23 ± 1 kg m^{-2}. Subjects in the BRE group were 32 ± 2 years, 174 ± 1 cm, 72 ± 2 kg and had a BMI of 24 ± 1 kg m^{-2}. The bed-rest investigation was conducted in the facilities at the Institute for Space Medicine and Physiology (MEDES) Clinic in Toulouse, France.

Each volunteer was interviewed and had a general physical examination that included blood and urine chemistries. Trained personnel at the MEDES clinic performed all screening of the potential volunteers. Prior to the screening, all potential subjects were informed of all procedures and risks associated with the experimental testing. Informed consent was obtained from each volunteer. The study was conducted in accordance with the Declaration of Helsinki.

Whole muscle strength and size

To document muscle strength and size changes in response to the intervention protocols, isometric and dynamic muscle tests were performed before and after bed-rest in both groups. Magnetic resonance imaging (MRI) was conducted to determine muscle cross-sectional area (CSA) of the thigh muscles before and after bed-rest in both groups.

Muscle strength measurements. All muscle strength profiles were conducted on a specially designed flywheel ergometer (Berg & Tesch, 1994, 1998; Tesch *et al.* 2003) that was adapted for the 6 deg head-down tilt experiments (Alkner & Tesch, 2004). Measurements were obtained four times prior to bed-rest and on day 90 of bed-rest prior to reambulation. Maximal isometric squats with a knee angle of 90 deg and two sets of seven maximal concentric and eccentric actions were performed. Subjects were instructed to push with maximal force in the concentric phase, wait a brief moment just after the rotational turning point of the flywheel, then brake (i.e. eccentric phase) until reaching approximately 70 deg of knee angle. The

ergometer was interfaced to a personal computer with customized software (MuscleLab, Ergotest AS, Langesund, Norway). Data were collected at 100 Hz. Dynamic test data consisted of concentric and eccentric peak force and concentric peak power. The data were averaged across repetitions for the set with the greatest average force.

MRI measurements. Muscle cross-sectional area (CSA) was obtained before bed-rest and on day 89 of bed-rest. Following 1 h of supine rest to control for the influence of postural-related fluid shifts on muscle size (Berg *et al.* 1993), MRI measurements were obtained for each subject. Subjects were supine and their heels were fixed on a non-metallic support to control joint and scan angle and to minimize compression of the legs against each other and the MRI gurney. Imaging was completed in a 1.0 T GE Siemens scanner (Somatam Impact, Erlangen, Germany) to determine the cross-sectional area (CSA) of the total quadriceps femoris, rectus femoris (RF), vastus lateralis (VL), vastus intermedius (VI), and vastus medialis (VM). Following the scout scans, interleaved transaxial images of 0.8 cm thick (TR/TE = 2000/20.0 ms, field of view 48 cm, 256 × 256 matrix) were taken from the top of the greater trochanter of the femur to the articular surface of the tibia.

MR images were transferred electronically from the scanner to a personal computer and analysed with Scion Image software (version 4.02) using manual planimetry. Analyses of the MR images began with the first proximal slice not containing gluteal muscle and continued distally to the last slice containing RF (Castro *et al.* 1999), because this region has been shown to represent the maximal CSA of the thigh (Narici *et al.* 1989). Muscle volume (MV; cm^3) was taken as the summation of all the analysed slices for an individual muscle and determined for the RF, VL, VI, and VM and summed for the total quadriceps femoris.

Resistance training protocol

The BRE group trained on the flywheel ergometer every third day (2–3 days per week) beginning on day 5 of bed-rest. Each exercise session consisted of four sets of seven (4 × 7) maximal repetitions employing the supine (6 deg head-down tilt) squat exercise. Two minutes of rest was allowed between sets. Force, flywheel rotational velocity (work and power were calculated) and the knee joint angles were recorded during each training session. The device is described in detail by Alkner & Tesch (2004).

Muscle biopsy

A muscle biopsy (Bergstrom, 1962) from the vastus lateralis of the dominant leg was obtained from each subject prior to bed-rest and again on day 84 of bed-rest (prior to reambulation). The muscle biopsy was performed on day 84 to avoid interference with several other testing procedures that were being performed during the final week of bed-rest prior to reambulation of the subjects.

A portion of each muscle sample was sectioned longitudinally into several pieces, placed in cold skinning solution (see below) and stored at −20°C for later analysis of single muscle fibre physiology. Following a single muscle fibre experiment, each single fibre was analysed for myosin heavy chain (MHC) composition as described below. Following each muscle biopsy, all single fibre contractile measurements were completed within a 4 week period.

Skinning, relaxing and activating solutions

The skinning solution contained (mM): 125 potassium propionate, 2.0 EGTA, 4.0 ATP, 1.0 $MgCl_2$, 20.0 imidazole (pH 7.0), and 50% (v/v) glycerol. The compositions of the relaxing and activating solutions were calculated using an iterative computer program described by Fabiato and Fabiato (1979). These solutions were adjusted for temperature, pH, and ionic strength using stability constants in the calculations (Godt & Lindley, 1982). Each solution contained (mM): 7.0 EGTA, 20.0 imidazole, 14.5 creatine phosphate, 1.0 free Mg^{2+}, 4.0 free MgATP, KCl and KOH to produce and ionic strength of 180 mM and a pH of 7.0. The relaxing and activating solutions had a free [Ca^{2+}] of pCa 9.0 and pCa 4.5, respectively (where pCa = −log Ca^{2+} concentration).

Single muscle fibre physiology experiments

On the day of an experiment, a 2–3 mm muscle fibre segment was isolated from a muscle bundle and transferred to an experimental chamber filled with relaxing solution where the ends were securely fastened between a force transducer (model 400A, Cambridge Technology, Watertown, MA, USA) and a DC torque motor (model 308B, Cambridge Technology) as described by Moss (1979). The instrumentation was arranged so that the muscle fibre could be rapidly transferred back and forth between experimental chambers filled with relaxing or activating solutions. The apparatus was mounted on a microscope (Olympus BH-2, Japan) so that the fibre could be viewed (× 800) during an experiment. Using an eyepiece micrometer, sarcomeres along the isolated muscle segment length were adjusted to 2.5 μm. All single muscle fibre experiments were performed at 15°C.

Unamplified force and length signals were sent to a digital oscilloscope (Nicolet 310, Madison, WI, USA) enabling muscle fibre performance to be monitored

throughout data collection. Analog force and position signals were amplified (Positron Development, Dual Differential Amplifier, 300-DIF2, Ingelwood, CA, USA), converted to digital signals (National Instruments, Inc.) and transferred to a computer (Micron Electronics, Nampa, ID, USA) for analysis using customized software. Servo-motor arm and isotonic force clamps were controlled using a computer interfaced force (position controller (Positron Development, Force Controller, 300-FC1).

For each single muscle fibre experiment, a fibre with a compliance (calculated as fibre length divided by y-intercept) greater than 10%, and/or a decrease in peak force of more than 10% was discarded and not used for analysis. The within-fibre test/re-test of a single muscle fibre in our laboratory for the measurements of size, force–power relationships, peak force and contractile velocity were less than 1%. The coefficients of variation for the force transducer and servo-mechanical lever mechanism during the 1 year period we examined single muscle cell function from the volunteers as part of this investigation were 0.4 and 0.6%, respectively.

Single muscle fibre analysis. Individual muscle fibres were analysed for diameter, peak force (P_o), maximal unloaded shortening velocity (V_o), and force–power characteristics. Detailed descriptions and illustrations of these procedures have been presented in our previous work (Trappe *et al.* 2003).

Single fibre diameter. A video camera (Sony CCD-IRIS, DXC-107 A, Japan) connected to the microscope and interfaced to a computer allowed viewing on a computer monitor and storage of the digitized images of the muscle fibres. Fibre diameter was determined from a captured computer image taken with the fibre briefly suspended in air (<5 s). Fibre width (diameter) was determined at three points along the segment length of the captured computer image using public domain software (NIH Image v1.61). Fibre cross-sectional area was calculated from the mean width with the assumption that the fibre forms a cylindrical cross-section when suspended in air.

Single fibre P_o. The outputs of the force and position transducers were amplified and sent to a microcomputer via a Laboratory-PC +12-bit data acquisition board (National Instruments, Inc.). Resting force was monitored and then the fibre was maximally activated in pCa 4.5 solution. Peak active force (P_o) was determined in each fibre by computer subtraction of the force baseline from the peak force in the pCa 4.5 solution.

Single fibre V_o. Fibre V_o was measured by the slack test technique as previously described (Edman, 1979). The fibre was fully activated in pCa 4.5 and then rapidly released to a shorter length, such that force fell to baseline. The fibre shortened, taking up the slack, after which force began to redevelop. The fibre was then placed in relaxing solution and returned to its original length. The duration of unloaded shortening, or time between onset of slack and redevelopment of force, was determined by computer analysis. Four to six different activation and length steps (each $\leq$15% of fibre length (FL)) were used for each fibre, with the slack distance plotted as a function of the duration of unloaded shortening. Fibre V_o (FL s^{-1}) was calculated by dividing the slope of the fitted line by the segment length and the data were normalized to a sarcomere length of 2.5 μm.

Single fibre power. Submaximal isotonic load clamps were performed on each fibre for determination of force–power parameters. Each fibre segment was fully activated in a pCa 4.5 solution and then subjected to a series of isotonic load steps. This procedure was performed at various loads so that each fibre was subjected to a total of 15–18 isotonic contractions.

For the force–velocity relationships, load was expressed as P/P_o, where P is the force during load clamping, and P_o is the peak isometric force developed prior to the submaximal load clamps. Force and shortening velocity data points derived from the isotonic contractions were fitted using the hyperbolic Hill equation (Hill, 1938). Only individual experiments in which r^2 was greater than or equal to 0.98 were included for analysis.

Fibre power was calculated from the fitted force–velocity parameters (P_o, V_{max} and a/P_o, where a is a constant). Absolute power (μN FL s^{-1}) was defined as the product of force (μN) and shortening velocity (FL s^{-1}). Normalized power (W l^{-1}) was defined as the product of normalized force (i.e. fibre force per cross-sectional area) and shortening velocity.

MHC determination

Following the single muscle fibre physiology measurements, each fibre was solubilized in 80 μl of 10% SDS sample buffer and stored at -20°C until assayed (Giulian *et al.* 1983; Williamson *et al.* 2001). In order to determine the myosin heavy chain (MHC) composition, fibres were run on a Hoefer SE 600 gel electrophoresis system that consisted of a 3.5% (w/v)

Table 1. Example calculations for the composite contractile velocity (V_o) variable from a bed-rest subject (BR group) after the 84 day intervention period

Variable	MHC I	MHC I/IIa	MHC IIa	MHC IIa/IIx	MHC I/IIa/IIx	Totals
Fibre number	6	5	4	7	3	25
Average V_o (FL s^{-1})	1.04	2.04	3.67	4.50	1.73	—
Total fibre V_o*	6.24	10.20	14.68	31.50	5.19	67.81
Composite V_o (FL s^{-1}) **	—	—	—	—	—	2.71

* Total fibre V_o calculated by multiplying fibre number by average V_o. **Composite V_o calculated by dividing total fibre V_o (67.81) by the total number of fibres (25).

acrylamide stacking gel with a 5% separating gel at 4°C. Following the gel electrophoresis, the gels were silver-stained as described by Giulian *et al.* (1983).

Statistical analysis and calculations

A two-way (group × time) ANOVA with repeated measures was used to determine if there were differences within and between groups for each of the following variables: whole muscle strength and size, single fibre diameter, P_o, P_o/CSA, V_o, V_{max}, peak power, normalized peak power and a/P_o. The number of studied fibres for an individual was averaged to represent a mean for MHC I and MHC IIa fibres. Significance was set at $P < 0.05$ and a Bonferroni *post hoc* test was used when significance was noted. All data are presented as means ± S.E.M.

Due to the low number of hybrid fibres studied before the bed-rest period, statistics were not performed on these fibre populations. However, these data are presented since these fibres do constitute a significant portion of the muscle profile following bed-rest.

Single fibre composite values. Due to the high proportion of hybrid fibres (which have a relative difference in their functional profile compared to fibres containing only one MHC isoform) after the bed-rest period, we implemented a weighted single fibre value system based upon the composition of the fibres studied from each subject. The resulting single fibre value for a given variable represents a 'composite' value that theoretically reflects the average for the entire muscle (in this case the vastus lateralis). For an example see Table 1. Combining all fibre types together, a pre-bed-rest composite V_o value would be 2.71 FL s^{-1} for this subject. Thus, the value 2.71 FL s^{-1} represents an average contractile speed for all the muscle fibres.

We recognize that these calculations are theoretical and make the assumption that the MHC profile of the fibres we randomly studied from the vastus lateralis of our subjects is representative of the whole thigh (quadriceps femoris). In support of this assumption, we did perform a more comprehensive MHC analysis from these same muscle biopsies (Harber *et al.* 2003), which is in close agreement with the MHC profile presented here. Using these composite estimates, we were able to include the large increase in hybrid fibres in the bed-rest analysis in an attempt to make a link between the myocellular functional results and the whole muscle results in this investigation. This procedure was conducted for the variables of diameter, P_o, P_o/CSA, V_o, power and normalized power for each individual and then averaged to represent the BR and BRE groups.

Results

Whole muscle size and function

Whole muscle size and functional data for the BR and BRE groups are shown in Table 2. Whole muscle size was reduced by 17% ($P < 0.05$) in the BR group and maintained in the BRE group. Isometric and isotonic measures of muscle function were reduced, on average, by 40% or more in the BR group. All indices of muscle function in the BR group showed significant losses compared to pre-bed-rest values. Conversely, the BRE group showed no significant decline in any of the isometric or isotonic muscle measurements.

Single muscle fibre MHC composition

The MHC profile is shown in Table 3. A total of 528 fibres were used for analysis in this investigation. Prior to the bed-rest period, both the BR and BRE groups had minimal hybrid fibres (13–14%) identified from the analysis. After the bed-rest period, the number of hybrid fibres (MHC I/IIa, IIa/IIx, or I/IIa/IIx) in the BR group increased ($P < 0.05$) to 49%. For the BR group, this appeared to be a fairly even distribution among the different hybrid types, with each type (I/IIa, IIa/IIx, I/IIa/IIx) contributing 15–17% to the total fibre population. In the BRE group, the total hybrid fibre population also increased ($P < 0.05$),

Table 2. Whole muscle quadriceps size and function from BR and BRE subjects before (Pre) and after (Post) 84 days of bed-rest

	BR group			BRE group		
Variable	Pre	Post	%Δ	Pre	Post	%Δ
MV (cm^3)	928 ± 47	770 ± 40*	−17	1111 ± 60	1105 ± 60	0
MVC (N)	1564 ± 191	876 ± 116*	−43	1388 ± 67	1216 ± 95	−11
CPF (N)	1628 ± 57	969 ± 59*	−41	1427 ± 121	1442 ± 167	+4
EPF (N)	1468 ± 49	937 ± 58*	−36	1433 ± 152	1299 ± 145	−7
PP (W)	704 ± 90	365 ± 51*	−47	530 ± 42	568 ± 67	+9

%Δ, percentage change. *$P < 0.05$ from Pre. MV, muscle volume; MVC, maximal voluntary contraction; CPF, concentric peak force; EPF, eccentric peak force; PP, peak power.

Table 3. Single fibre myosin heavy chain (MHC) composition of vastus lateralis fibres used in physiological experiments from BR and BRE subjects before and after 84 days of bed-rest

Group		MHC I	MHC I/IIa	MHC IIa	MHC IIa/IIx	MHC I/IIa/IIx	Total *n*
BR	Pre	73 (64%)	8 (7%)	26 (23%)	7 (6%)	0 (0%)	114
	Post	51 (35%)	23 (15%)	25 (17%)	26 (17%)	23 (16%)	148
BRE	Pre	58 (54%)	2 (2%)	34 (31%)	14 (13%)	0 (0%)	108
	Post	53 (35%)	32 (21%)	51 (34%)	15 (10%)	0 (0%)	151

For each MHC type the number of fibres studied is shown with the percentage breakdown in parentheses.

to 31%, which was less ($P < 0.05$) than the increase in the BR group. The increase in hybrid fibres in the BRE group was most prominent in the MHC I/IIa fibre type, with no MHC I/IIa/IIx fibres identified in the pre- or post-bed-rest samples of this group. In the BR and BRE groups the increases in hybrid fibres resulted in decreases ($P < 0.05$) of 29 and 19% in MHC I fibres, respectively.

Single muscle fibre diameter

Pre- and post-bed-rest fibre diameters are shown in Table 4. MHC I and IIa fibres were 15 and 8% smaller, respectivley, ($P < 0.05$) after the bed-rest period in the BR group. The BRE group did not show any significant changes in MHC I and IIa fibre diameters following bed-rest. After the bed-rest period, the MHC IIa fibres from the BR group were smaller ($P < 0.05$) compared to the BRE group.

Single muscle fibre P_o and P_o/CSA

Peak force and peak force per cross-sectional are shown in Table 4. As a result of the bed-rest, the BR groups' MHC I fibres showed a 47% decrease ($P < 0.05$) in P_o, while the MHC IIa fibres showed a 25% decrease ($P < 0.05$) in P_o. When corrected for cell size, the MHC I fibres still showed a decline ($P < 0.05$), suggesting that changes in fibre size could not explain all of the loss in peak force in these fibres. In contrast, MHC I and IIa fibres from the BRE group did not have any statistical reductions in P_o or P_o/CSA. While not significant, the MHC I fibres of the BRE group showed a trend for P_o ($P = 0.08$) and P_o/CSA ($P = 0.10$) to be lower.

Single muscle fibre shortening velocity

Contractile velocity of the muscle fibres was assessed using the slack test procedure (V_o) and the force–velocity procedure (V_{max}) (see Methods). Both of these measurements provided a measure of shortening velocity and are shown in Table 5. The MHC I fibres had 21 and 22% drops ($P < 0.05$) in fibre V_o and V_{max}, respectively, in the BR group. No change in MHC IIa fibre contractile velocity was found in the BR group. Likewise, no change in contractile velocity of the MHC I or IIa fibres was observed in the BRE group after bed-rest.

Single muscle fibre power

Absolute peak power and peak power normalized for muscle cell size of the MHC I and IIa fibres for the BR and BRE groups are shown in Table 6. The MHC I fibres from the BR group showed a 55% reduction ($P < 0.05$) in peak power after bed-rest. When adjusted for cell size, normalized peak power was still reduced ($P < 0.05$) by 41% in the MHC I fibres from the BR group. No significant reductions were observed in the MHC IIa fibres from the BR group as a result of the bed-rest. However, a trend

Table 4. Single muscle fibre diameter, peak force (P_o) and specific force (P_o/CSA) for all vastus lateralis myosin heavy chain (MHC) types from BR and BRE subjects before (Pre) and after (Post) 84 days of bed-rest

	MHC I		MHC I/IIa		MHC IIa		MHC IIa/IIx		MHC I/IIa/IIx	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Diameter (μm)										
BR	91 ± 3	77 ± 5*	93 ± 5	80 ± 4	95 ± 5	87 ± 5†	86 ± 6	86 ± 4	N/A	75 ± 2
BRE	87 ± 5	91 ± 5	102 ± 8	87 ± 4	97 ± 4	105 ± 8	88 ± 7	108 ± 9	N/A	N/A
P_o (mN)										
BR	0.65 ± 0.04	0.34 ± 0.05*	0.69 ± 0.09	0.42 ± 0.05	0.88 ± 0.08	0.66 ± 0.07*	0.77 ± 0.07	0.61 ± 0.07	N/A	0.41 ± 0.08
BRE	0.63 ± 0.06	0.51 ± 0.04	0.91 ± 0.16	0.63 ± 0.04	0.93 ± 0.10	0.97 ± 0.10	0.85 ± 0.11	0.84 ± 0.16	N/A	N/A
P_o/CSA (kN m^{-2})										
BR	101 ± 12	73 ± 9*	103 ± 14	85 ± 12	124 ± 12	108 ± 9	158 ± 16	105 ± 10	N/A	89 ± 16
BRE	104 ± 5	83 ± 11	116 ± 37	106 ± 14	129 ± 14	117 ± 15	141 ± 10	90 ± 7	N/A	N/A

*$P < 0.05$ from Pre. †$P < 0.05$ between groups.

Table 5. Single muscle fibre shortening velocity using the slack test procedure (V_o) and force–velocity procedure (V_{max}) for all vastus lateralis myosin heavy chain (MHC) types from BR and BRE subjects before (Pre) and after (Post) 84 days of bed-rest

	MHC I		MHC I/IIa		MHC IIa		MHC IIa/IIx		MHC I/IIa/IIx	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
V_o (FL s^{-1})										
BR	1.18 ± 0.08	0.93 ± 0.09*	2.43 ± 0.61	1.86 ± 0.27	3.80 ± 0.44	3.58 ± 0.35	4.71 ± 0.62	4.24 ± 0.20	N/A	1.99 ± 0.39
BRE	1.30 ± 0.09	1.29 ± 0.06	2.98 ± 1.44	2.41 ± 0.50	3.75 ± 0.42	4.23 ± 0.33	4.78 ± 0.44	4.38 ± 0.17	N/A	N/A
V_{max} (FL s^{-1})										
BR	0.72 ± 0.11	0.56 ± 0.06*	1.92 ± 0.62	0.90 ± 0.20	3.31 ± 0.36	2.23 ± 0.28	4.22 ± 0.71	3.56 ± 0.18	N/A	1.55 ± 0.54
BRE	0.67 ± 0.10	0.71 ± 0.10	1.85 ± 0.94	1.57 ± 0.31	2.92 ± 0.48	3.13 ± 0.22	4.22 ± 0.17	3.88 ± 0.27	N/A	N/A

*P < 0.05 from Pre.

Table 6. Single muscle fibre peak power (Absolute power) and peak power normalized to cell size (Norm power) for all vastus lateralis myosin heavy chain (MHC) types from BR and BRE subjects before (Pre) and after (Post) 84 days of bed-rest.

	MHC I		MHC I/IIa		MHC IIa		MHC IIa/IIx		MHC I/IIa/IIx	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Absolute power (μN FL s^{-1})										
BR	11.6 ± 1.2	5.2 ± 0.8*	28.1 ± 8.2	11.5 ± 3.4	59.2 ± 8.2	44.7 ± 6.0	81.8 ± 11.2	44.9 ± 3.5	N/A	17.82 ± 8.41
BRE	12.8 ± 1.7	9.1 ± 0.8*	50.0 ± 32.9	23.3 ± 4.8	68.2 ± 9.3	68.9 ± 7.1	86.2 ± 11.2	88.7 ± 17.3	N/A	N/A
Norm power (W l^{-1})										
BR	1.89 ± 0.29	1.12 ± 0.13*	3.84 ± 0.97	2.41 ± 0.76	8.28 ± 7.16	7.16 ± 0.51	16.84 ± 2.90	7.91 ± 0.72	N/A	4.07 ± 1.91
BRE	2.14 ± 0.17	1.47 ± 0.20*	6.82 ± 5.03	3.97 ± 1.00	9.56 ± 1.29	8.26 ± 1.05	14.42 ± 1.29	9.39 ± 0.15	N/A	N/A

*$P < 0.05$ from Pre.

($P = 0.10$; –25%) towards lower peak power values was observed in the MHC IIa fibres from the BR group.

The BRE group showed a 29% decline ($P < 0.05$) in MHC I fibre peak power. When adjusted for cell size, normalized power was still reduced ($P < 0.05$) in the MHC I fibres of the BRE group after bed-rest. No significant alterations were observed for the MHC IIa fibres of the BRE group following bed-rest.

Single muscle fibre composite data

Shown in Table 7 are the composite values for diameter, P_o, P_o/CSA, V_o, power and normalized power. The BR group had a reduction in all variables, with the exception of V_o. In contrast, the BRE maintained all parameters except for the normalized values of force and power.

Discussion

One of the main goals of our research team over the past several years has been to document the loss in size and function of human skeletal muscle at the whole muscle and cellular level with unloading and to identify appropriate exercise countermeasures to offset this loss. Currently, an optimal exercise programme for protecting the skeletal muscles of humans during long-term unloading has not been elucidated. This project was a first attempt to

Table 7. Single muscle fibre vastus lasteralis composite data (see Methods for description) from BR and BRE subjects before (Pre) and after (Post) 84 days of bed-rest

	BR group			BRE group		
Variable	Pre	Post	%Δ	Pre	Post	%Δ
Diameter (μm)	91 ± 3	82 ± 3*	−10	92 ± 4	97 ± 5	+5
P_o (mN)	0.71 ± 0.05	0.45 ± 0.03*	−36	0.69 ± 0.08	0.72 ± 0.05	+4
P_o/CSA (kN m^{-2})	109 ± 12	86 ± 6*	−21	116 ± 9	99 ± 11*	−15
V_o (FL s^{-1})	2.10 ± 0.13	2.34 ± 0.18	+11	2.51 ± 0.18	2.80 ± 0.28	+12
Power (W l^{-1})	27.9 ± 3.1	21.5 ± 1.8*	−23	39.3 ± 4.9	39.5 ± 4.6	0
NPower (kN m^{-2} FL s^{-1})	4.4 ± 0.6	3.9 ± 0.3	−11	5.9 ± 0.7	5.1 ± 0.6	−13

%Δ, percentage change. *$P < 0.05$ from Pre. P_o, peak force; P_o/CSA, force per cross-sectional area; V_o, unloaded shortening velocity; Power, absolute power; Npower, normalized power.

Table 8. Average nutritional daily intake information from the pre-bed-rest (15 day average), bed-rest (90 day average) and post-bed-rest (15 day average) phases for the BR and BRE groups

Group		Energy intake (kcal)	Carbohydrate (g)	Fat (g)	Protein (g)
BR	Pre	2355 ± 87	318 ± 14 (55%)	76 ± 3 (29%)	95 ± 2 (16%)
	Bed-rest	2083 ± 87	255 ± 15 (50%)	75 ± 3 (32%)	92 ± 2 (18%)
	Post	2222 ± 104	281 ± 21 (51%)	78 ± 2 (32%)	93 ± 2 (17%)
BRE	Pre	2504 ± 57	345 ± 9 (56%)	79 ± 2 (28%)	100 ± 1 (16%)
	Bed-rest	2215 ± 58	290 ± 8 (53%)	74 ± 2 (30%)	94 ± 3 (17%)
	Post	2474 ± 89	337 ± 12 (55%)	81 ± 4 (29%)	97 ± 4 (16%)

The numbers in parentheses represent the percentage contributions of each nutrient.

examine the possibility that high-intensity, low-volume resistance exercise is sufficient to maintain skeletal muscle mass and function during long-duration unloading. The main finding from this investigation is that the resistance exercise programme used during bed-rest was effective for maintaining whole muscle size and function of the thigh muscles. However, muscle biopsies from the vastus lateralis showed that slow- and fast-twitch single muscle fibre function, along with the MHC profile of individual muscle fibres, was differentially affected by bed-rest. These data show that: (1) the MHC IIa fibres were protected by the resistance exercise programme, (2) the MHC I fibres were partially protected by the resistance exercise programme, and (3) both the BR and BRE groups showed a significant increase in hybrid fibres, with BRE showing less of an increase compared to BR.

The single muscle fibre results are intriguing in that they appear to contrast the whole muscle findings. For the control group it was no surprise that both MHC I and IIa fibre functions were severely compromised as a result of the long-term bed-rest period (summarized in Figs 2 and 3). However, what was surprising was the reduction in some of the single cell functional parameters in the BRE group, despite the apparent maintenance of whole muscle size and function. The BRE MHC I fibres did maintain size and this, when coupled with the size preservation of the other fibre types in this group, provides an explanation of why whole muscle size was maintained. However, the BRE MHC I fibres showed a decline in the functional measures of strength, speed and power after bed-rest (Fig. 2). The BRE MHC IIa fibres maintained or increased cell size, peak force, contractile velocity, and power (Fig. 3). Although it appears that the exercise did have some positive benefit for the MHC I fibres, it was not sufficient to completely maintain single cell function compared to pre-bed-rest values.

With bed-rest, there was a large increase in the number of hybrid muscle fibres in both groups (Table 3). The BR group had an increase in hybrid fibres from 13 to 49%, while the BRE group had an increase in hybrid fibres from 14 to 31%. This shift in fibre composition was a directional shift from a slower contracting fibre to a faster contracting fibre. This slow-to-fast shift in myosin isoform composition is in agreement with previous unloading investigations in animals (Caiozzo *et al.* 1994, 1996) and humans (Zhou *et al.* 1995; Ohira *et al.* 1999). The MHC profile shift of the entire vastus lateralis would, in theory, lead to a faster contracting muscle. Or, in the case of this study, the shift in myosin isoform from slow to fast may have been sufficient to pre-serve whole muscle performance in the BRE group.

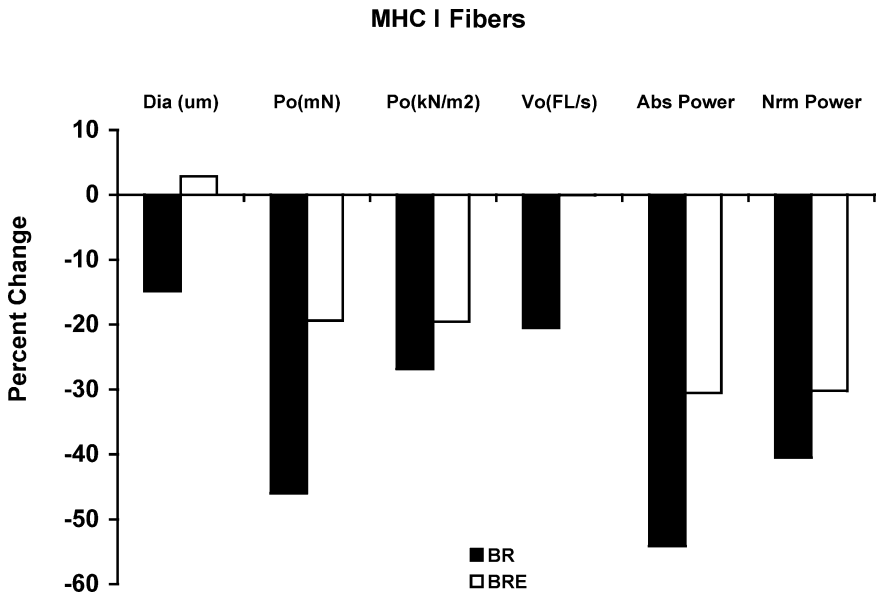

Figure 1. A summary of the single muscle fibre parameters for the MHC I fibres for subjects in the bed-rest only (BR) group and bed-rest + resistance exercise (BRE) group
For each variable the percentage change is shown.

A unique finding from this study was the rather large number of MHC I/IIa/IIx fibres that were present in the control group muscles after bed-rest. Before bed-rest, neither the BR nor the BRE group had any identifiable MHC I/IIa/IIx. After bed-rest, however, a total of 23 fibres (16%) that had all three isoforms present were identified from the BR group. This is unique in that this fibre type is rarely observed in human skeletal muscle. Larrson *et al.* (1996) did not report any fibres containing all three isoforms from the human vastus lateralis muscle after 37 days of bed-rest. In our previous studies with short-term spaceflight (Widrick *et al.* 1999), ageing (Trappe *et al.* 2000, 2001*b*, 2003) and athletes (Trappe *et al.* 2001*a*), this MHC triple-hybrid isoform fibre has not been observed. This is in agreement with the work of others that has profiled the functional characteristics of normal active human skeletal muscle (Bottinelli *et al.* 1996, 1999, 2001). The functional profile of this novel MHC isoform falls in the spectrum between the MHC I/IIa hybrid and the pure MHC IIa isoform. The MHC IIx component of the MHC I/IIa/IIx fibre slightly increased the shortening velocity and power component compared to the MHC I/IIa fibres. The addition of the IIx isoform to the MHC I/IIa fibre may play a key role in increasing the contractile speed of fibres falling in the moderate range of functionality. The finding that long-term unloading resulted in one-sixth of the vastus lateralis muscle to be composed of the novel triple-hybrid isoform is reported

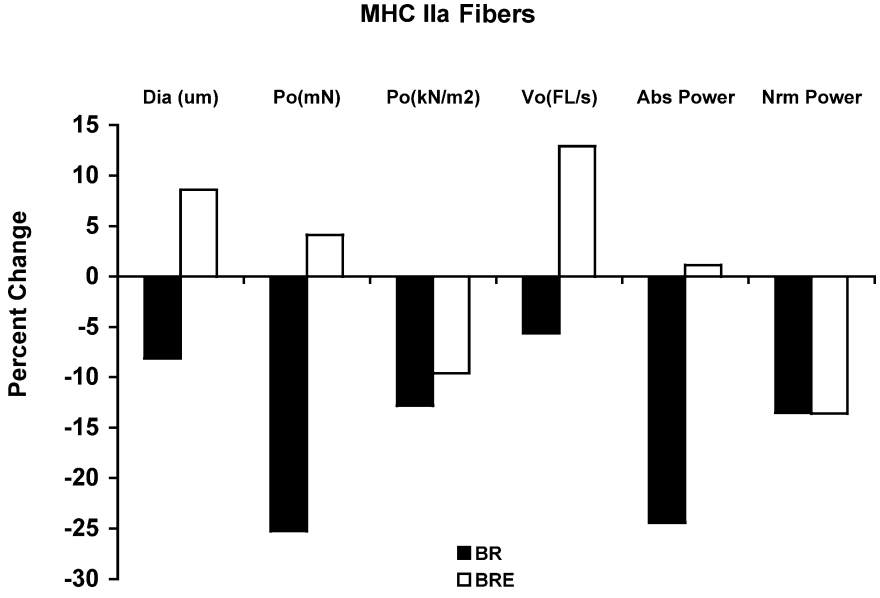

Figure 2. A summary of the single muscle fibre parameters for the MHC IIa fibres for subjects in the bed-rest only (BR) group and bed-rest + resistance exercise (BRE) group
For each variable the percentage change is shown.

here for the first time. The physiological role for this fibre type is presently unknown, as are the mechanisms that regulate selected muscle fibres to contain all three main isoforms in human skeletal muscle.

Most often single muscle fibre data are 'compartmentalized' into specific fibre types during analysis and when published. However, the vastus lateralis studied in this investigation was composed of a mixture of slow, fast, and a variety of hybrid muscle fibres, which comprise a continuum of functionality that contributes differentially to whole muscle performance. With this in mind, we wanted to provide an estimate of single muscle fibre function for all fibre types acting together (Table 7). This 'composite' value theoretically reflects the function of a given variable for multiple fibre types contracting together and provides some additional insight as to why whole muscle performance was unaffected in the BRE group. As can be seen in Table 7, the BR group had a reduction in single fibre size and all functional variables (except V_o), while the BRE group maintained most parameters. Of particular interest was single fibre power, since it is a product of strength and speed. The composite single muscle fibre power production was reduced by 23% in the BR group, but maintained in the BRE group, again providing additional information to help relate the single muscle fibre findings to whole muscle performance.

Another aspect worth noting was that the MHC I fibres appeared to be more affected in the control group compared to the MHC IIa fibres. This is in agreement with animal data showing that anti-gravity muscles that are composed primarily of slow-twitch fibres, such as the soleus, are more affected by unloading compared to muscles with a fast-twitch fibre component (Fitts *et al.* 2000). Following 5 weeks of bed-rest, LeBlanc *et al.* (1988) reported a 30% loss in human whole muscle peak torque of the calf and reported that whole muscle performance was less impacted at higher angular velocities. This also supports the idea that slow-twitch fibres appear to have a greater loss in function with unloading compared to fast-twitch fibres. From the current investigation, single muscle fibre power was reduced in the MHC I fibres by nearly 60%, and only about 25% in the MHC IIa fibres. This suggests that the lack of a 1 g load that is typically placed upon the muscles for normal daily activities (walking, simple tasks, posture, etc.) may play a bigger role in preserving these fibres than previously thought. As a result, resistance training alone may not be sufficient to preserve the contractile function of slow-twitch fibres during long-term unloading.

Single fibre data from vastus lateralis muscle have consistently shown that MHC IIa fibres produce 5- to 6-fold more power compared to MHC I fibres (Table 6; Trappe *et al.* 2000, 2003). In the current investigation, the MHC IIa fibres were ~5-fold more powerful compared to the MHC I fibres for both the BR and BRE groups prior to bed-rest. Following bed-rest, the power relationship between the MHC I and IIa fibres was altered in both groups. For the BR group the MHC IIa fibres were 8–9 times more powerful compared to the MHC I fibres after bed-rest. This ratio was slightly altered in the BRE group after bed-rest, with the MHC IIa fibres having a ~7-fold greater power output. This highlights the importance of the fast-twitch fibre types in their ability to generate power and their potential contribution to whole muscle power. Granted the MHC I fibres were more affected by bed-rest in both groups, but the decline in MHC I size, contractile speed and power would, in theory, have much less of an impact to a decline in whole muscle power output than if the MHC IIa fibres were not protected by exercise countermeasures. The maintenance of the MHC IIa power output in the BRE subjects of the current investigation would thus appear to be one of the major contributing factors to the similar whole muscle results pre- and post-bed-rest.

Single muscle fibre contractile velocity was reduced in the control group, which is both in contrast to (Widrick *et al.* 1997, 1999; Yamashita-Goto *et al.* 2001) and agreement with (Larsson *et al.* 1996; Widrick *et al.* 2002) previous human studies. With the exception of one investigation (Yamashita-Goto *et al.* 2001), studies with no exercise during the unloading period have all shown a decline in contractile speed at the single fibre level. Studies with designed countermeasures or mandated exercise programmes while in orbit have shown an elevation in single fibre contractile velocity (Widrick *et al.* 1997, 1999). Findings from the current investigation provide further evidence that during periods of unloading with no exercise single fibre contractile speed declines and when exercise countermeasure programmes are employed, single fibre contractile velocity is elevated. Thus, it does appear that muscle activity plays a significant role in modulating contractile velocity in each specific fibre type. As we (Trappe *et al.* 2000, 2001*b*) and others (Bottinelli, 2001) have reported, this alteration in contractile speed can occur without a change in MHC isoform. While the mechanism for this physiological alteration is unknown, several candidates such as undetected MHC isoforms (Bottinelli, 2001), alterations in myosin light chain composition (Bottinelli, 2001) or glycation of the myosin head (Ramamurthy *et al.* 2001) have been postulated.

Although each specific fibre type showed a decline or a tendency for a decline in fibre V_o for the control group, the composite V_o data in the control group indicated

an increase of 11%. This is in close agreement with the 12% increase in the composite single fibre contractile velocity for the exercise group. As stated earlier, both the BR and BRE groups had an increase in hybrid muscle fibres, resulting in a slow- to fast-fibre transition following the bed-rest period. When combining the fibre type shift information with the contractile velocity data it becomes apparent that each individual fibre type had a decrease in contractile velocity, but the overall contractile velocity profile of the vastus lateralis was faster due to the myosin isoform changes. While isolated individual muscle fibres provide important information about a specific fibre type, this information may be misleading from a more global or whole muscle perspective.

In animals using the hindlimb unloading model, *in vitro* preparations have consistently reported contractile velocity to be elevated (cf. Fitts *et al.* 2000). What is interesting about these data is that no exercise during the hindlimb unloading period still resulted in an increase in single fibre V_o, which is in contrast to human studies with no exercise during unloading. However, muscle activity, as measured by EMG activity, initially was reduced in these animals and then gradually increased with the period of unloading (Alford *et al.* 1987). This suggests that the muscle was not in a state of reduced contractile activity during unloading in animals, except for the first few days of hindlimb suspension, and may have contributed to the rise in V_o. In contrast, human EMG activity has been shown to be reduced by ∼35–40% after 90–180 days of unloading (Lambertz *et al.* 2001). These data provide some evidence that the electrical activity to the muscle may differ with unloading among animals and humans and therefore direct comparisons between species and models of unloading should be made with caution.

Previous bed-rest studies of 37 days (Larsson *et al.* 1996) and 4 months (Yamashita-Goto *et al.* 2001) have reported a decline in MHC I fibre peak force normalized to cell size (i.e. specific force) of ∼40%. Slow- and fast-twitch muscle fibres from the control subjects (BR group) in the current study showed declines in specific force of ∼25 and ∼14%, respectively. Shorter duration periods of unloading have shown no change in specific force with bed-rest (Widrick *et al.* 1997) or spaceflight (Widrick *et al.* 1999). Given that the latter two studies employed periodic testing during the unloading period, one might speculate that specific force may be maintained with unloading provided there is an exercise programme. However, the slow- and fast-twitch muscle fibres from the exercise group of the current investigation also showed a decline in specific force, indicating that unloading duration and not exercise *per se* may be a contributing factor in specific force alterations. Riley *et al.* have shown that the actin protein is preferentially lost compared with myosin with 17 days of spaceflight (Riley *et al.* 2002) and bed-rest (Riley *et al.* 1998), but this did not translate into a decline in specific force of fibres from the soleus (Widrick *et al.* 1997, 1999). Thus, some other aspect of the contraction process (i.e. alterations in the contractile or myofibrillar proteins, calcium kinetics, force per cross-bridge, etc.) must be occurring to influence force per unit area in single muscle fibres during periods of long-term unloading.

A deficit in the energy intake by humans during bed-rest or while in space poses an additional concern that has been linked to muscle wasting (Ferrando *et al.* 1996; Stein *et al.* 1999). The voluntary energy intake of crew members while in space has been reported to be reduced by ∼20% (Stein *et al.* 1999). In the current investigation, subjects were fed prepared meals by the staff that were nutritionally balanced. For both the BR and BRE groups, the amount of energy consumed along with the composition of carbohydrates, fats, and proteins was consistent before, during and after the bed-rest period (Table 8). Of particular importance was the protein intake, since this has been shown to be critical for the anabolic and catabolic aspects of muscle protein turnover (Ferrando *et al.* 2002). Subjects from the current investigation consumed, on average, 90–100 g of protein per day, which is equivalent to 1.2–1.4 g (kg body weight)$^{-1}$. This level of protein intake is in excess of the current recommended daily allowance (RDA) in the United States (0.8 g (kg body weight)$^{-1}$) and within the range recommended for space travellers (Stein, 1994). Thus, it does not appear that the muscle alterations observed in the current investigation can be directly attributed to poor nutrition.

In summary, this investigation demonstrated that high-intensity, low-volume resistance exercise every 2–3 days was effective for maintaining whole muscle strength and size during long-term bed-rest. Physiological studies on single muscle fibres from the vastus lateralis showed that the contractile function was preserved in the fast-twitch fibres, but not in the slow-twitch fibres from subjects who performed the resistance exercise. This discrepancy between the *in vivo* (whole muscle) and *in vitro* (single muscle fibre) studies appears to be related, in part, to the increase in hybrid muscle fibres and the relative functional contribution of the different fibre types. For the BRE group, the shift away from the slower less powerful fibres to faster more powerful fibres (MHC I → MHC I/IIa) coupled with the 5-fold greater power output of the fast-twitch fibres compared to the

slow-twitch fibres, was sufficient to maintain whole muscle function. In light of the inability of the resistance training to maintain the functional characteristics of the slow-twitch fibres, additional exercise interventions that target and provide adequate loading for this fibre type should be considered.

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