SINGLE SCAN



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**Session JA3**
**Room 3**
**8:30 - 11:30 a.m.**

# Effect of Real and Simulated Microgravity on Muscle Function

# CHANGES IN CALF MUSCLE PERFORMANCE, ENERGY METABOLISM, AND MUSCLE VOLUME CAUSED BY LONG TERM STAY ON SPACE STATION MIR

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## INTRODUCTION

The decrease in performance of anti gravitational muscles due to space flight conditions has been studied by several groups of scientists in terms of tissue mass, muscle performance, and neuro-muscular characteristics (1, 2, 3). However, the role of changes in energy metabolism, determined by the ratio of oxidative and glycolytic muscle fibres and their densities in mitochondria and glycolytic enzymes, is still unclear. For this reason, we have used $^{31}P$ magnetic resonance spectroscopy to monitor the energy metabolism in resting, working, and recovering calf muscle before and after space flight in the scope of the space missions EuroMIR'94 and '95.

## SUBJECTS AND METHODS

3 to 6 times pre-flight and 3 times post flight (return +4,+9 to 11, and +30 days) examinations were performed on the right lower leg of one cosmonaut, who was on Mir for one month, and on three cosmonauts, who stayed on Mir for 6 months. Space flight conditions are characterized by unloading due to microgravity and by the Russian countermeasure program containing treadmill and velo ergometer exercise for approximately 2 hours per day. The cross-sectional areas and the volumes of the plantar flexors were determined from T1-weighted magnetic resonance images acquired at 1.5 Tesla. Energy metabolism was monitored by $^{31}P$-MR spectroscopy at 4.7 or 1.5 Tesla, respectively. Spectra were obtained in intervals of 20 s during an exercise protocol containing three periods of 3 min isometric foot plantarflexion with increasing muscle tension (20, 40, and 60% of current maximum voluntary contraction force, MVC) on a pedal ergometer each followed by 5 min of recovery. Spectra were analysed for phosphomonoesters (e.g. glucose-6-phosphate), inorganic phosphate, phosphocreatine (PCr), adenosine trisphosphate (ATP), and intracellular pH. The time constant of PCr repletion in recovery from exercise was assessed in order to characterize the oxidative metabolism in calf muscle (4). Since muscle forces equal to, or higher than, 60% MVC cause a functional ischemia, the overall energy turnover was calculated from the rate of PCr depletion at the very onset of contraction. Glycolytic ATP formation was calculated from the difference between the initial and final rates of PCr depletion 60% MVC (5).

## RESULTS

In all cosmonauts volumes of plantar flexors in the calf were decreased by 6 to 20%, as compared to average pre-flight values. MVC was unchanged after one months in space but decreased by 20% to 48% after 6 months. Reductions in force could not be correlated with the decrease in muscle volumes.

After return from a 1 month space flight all three exercise steps were executed as nominally required. The oxidative PCr recovery after contraction was delayed and glycolytic ATP formation at high forces (60% MVC) was reduced.

After 6 months in space, the 60% MVC step of isometric contraction test was terminated after 1 to 1.5 min, instead of the demanded 3 min, because of muscle pain. However, pain occurred after little exercise in absolute terms resulting in only small depletion of PCr stores by 20% to 65% and an intracellular acidification which was less than 0.1 to 0.2 pH-units. PCr and pHi kinetics did not indicate changes in the capacities of oxidative and glycolytic metabolism compared with pre flight conditions. However, in subject metabolic efficiency (total ATP turnover versus force time integral) was decreased by 33%. About 1 month post flight all investigated parameters had returned to normal in all subjects, independent from flight duration.

## DISCUSSION

Post-flight changes in aerobic and anaerobic work capacities were only observed in one subject who was in space for 1 month.. However, after 6 months in space, 3 subjects did not show any change in their aerobic and anaerobic metabolic capacities. The single case after one month in space might be representing a transition state during long term space flight or was more probably due to intense exercise one day before examination. This will be studied in

the scope of the German - Russian space mission MIR'97. Furthermore, in 1997, present findings for a 6 months long space flight will be proved on further 3 subject in the scope of MIR'97 and EuroMIR-E.

The decrease in calf muscle performance observed in early post-flight examinations in EuroMIR'94/ '95 cosmonauts cannot only be explained on the basis of a decrease in muscle volume. Furthermore, early painful fatigue due to isometric contraction, which occurred after 6 months in space, was clearly not induced by energy depletion or acidification. Effects of space flight conditions on the capacities of energy metabolism are of little or no physiological relevance for the reduction in muscle performance. Although long term static contraction of the calf muscles did almost not occur under microgravity, endurance kinetic exercise e.g. on the treadmill seemed to be sufficient to conserve the relative fraction of red muscle fibres.

The decrease in maximum and endurance muscle performance after 6 months space flight may result from a decrease in the solidity of the muscle tissue and in consequence a different mechanical behaviour during contraction detected by mechanoreceptors.

## OUTLOOK

In the scope of MIR'97 and EuroMIR-E we will examine passive and active fluid shifts in resting and exercising calf muscles using MRI in combination with a lower leg differential pressure device. This device simulates fluid shifts which are normally driven by the gravitational field and different body positions. A decrease in muscle solidity will be determined as an increase in fluid uptake of resting muscle tissue at a given pressure. First pre-flight results have shown that at negative pressure the volume of muscle tissue is swelling almost to the same extent as the whole lower leg. This means, on earth distinct fluid shifts do not only occur in subcutaneous tissues but to the same extent in muscle. As a powerful tool MRI will contribute to this systemphysiological approach on muscle physiology.

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# VIBROGRAFIC SIGNS OF AUTONOMOUS MUSCLE TONE STUDIED IN LONG TERM SPACE MISSIONS

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## INTRODUCTION

Questions concerning the regulation of autonomous mucle tone in relaxed and postural states of the human peripheral motor system are still a matter of debatte. Basmajian/de Luca [1] for example found no myografic evidence for muscle activity in relaxed limbs and Lakie et al [2] found that stiffening in a relaxed muscle is produced by a thixothropic behaviour of muscle tissue. On the other hand additional muskuloskeletal relaxation was found in REM sleep [3], and cardiac produced 'microvibrations' (MV) of the body showed sensitve to the direction of optokinetic stimulation [4], indicating a mechanism underlying muscle tone. It was therefore of interest to study signs of muscle tone in the absence of gravitational forces as well as after a long term flights during the readaptation phase.

## METHODS

Muscle tone of the arm was assessed by vibrografic signals (accelerometers) in six cosmonauts staying 4 - 6 months in space and in one cosmonaut staying 14 months in space. In the relaxed (free floating) state of the body microvibrations (MV) over muscle tissue (M. brachioradialis) were investigated. The experimental protocol was: maximal relaxation, activation of respiratory muscles and slightly extending the arm. In the postural states of the arms physiological tremor (PT) was studied over bony tissue. The experimental protocol was: keep the arm forward, close eyes, reach both hands and use an elastic band to load both arms isotonically. To determine the relation of the vibrografic signals to the cardiac cycle an electrocardiagram was recorded simultaneously.

## RESULTS

Accelerometric recorded forearm MV in 1 g showed the typical 7-13 Hz oscillations triggered by the heart beat. In 0 g, during the fully relaxed state, these oscillations were decreased in amplitude and shifted to lower frequency. Activation of respiratory muscles had little effect, whereas slight extension of the arm in 0 g resulted in a similar MV amplitude and frequency as in 1 g during the relaxed state. No postflight effects were found. PT showed an irregular waveform in 1 g. In 0 g tremor amplitude was reduced and postflight the amplitude was increased in relation to preflight. Closure of eyes and reaching both arms showed no effect, whereas stretching of the elastic band produced large tremor oscillations postflight. Further the resistance to fatigue was reduced postflight.

## CONCLUSION

Signs of muscle tone in the relaxed state (MV) were generally decreased in 0 g, but when muscle tone was produced voluntary in the 0 g environment a similar MV occurs as in 1 g. This indicates that actually some kind of resting muscle tone is present in 1 g, producing the 7 -13 Hz component of MV. Multijoint musculoskeletal stiffness is proposed as mechanism to convert resting muscle tone into 7-13 oscillations in response to the cardiac impact.
The reason for the decrease of tremor amplitude in 0 g is mainly given by the fact that no muscular contraction is necessary to compensate gravitational forces. The increases of tremor amplitude postflight, indicates a reduction of stability in the motor system. At the moment it is unclear wheather periperal or central adaptation processes are responsible for these effects.

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# REDUCTION OF MUSCLE STRENGTH AFTER LONG DURATION SPACE FLIGHTS IS ASSOCIATED PRIMARILY WITH CHANGES IN NEUROMUSCULAR FUNCTION.

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## INTRODUCTION

Decreases in muscle strength after long duration space flight could result from alterations in neural control. Siconolfi et al. (1996) reported increases in the electromyographic response during maximal contractions after the Mir 18 flight (115 days). Koryak et al. (1997) reported that one subject had 8% and 35% decreases in maximal tetanic and voluntary contractions, respectively, after the 115 days of the Mir 18 flight. Siconolfi et al (1997) reported decreases in peak running speed 5-6 days after the Mir 18 flight while oxygen uptake was not changed. Peak running speed returned to near preflight levels 18-19 days after return to earth. This study presents the integration of these reports with the post-flight decreases in peak (of 3 contractions) concentric knee strength at different velocities (below) in 5 crewmembers from Mir 18 and 19 (77 days) space flights.

## METHODS

Strength was assessed on a LIDO Multi-joint Isokinetic Dynamometer. Crewmembers performed various levels of in-flight exercise. One crewmember (C) perfomed minimal exercise during flight while the others exercised on a treadmill, cycle with or without resistance exercise. Two of the exercising crewmembers completed 70% and 90% of the prescribed treadmill exercise (TE) protocol but only at <70% of the recommended exercise intensity. The other two crewmembers performed treadmill, bicycle and resistive exercise (TBRE) at >80% of prescribed exercise volume and intensity.

## RESULTS

| | C-Mir 18 | | 70%TE-Mir 18 | | 90%TE-Mir 18 | | TBRE-Mir 19 | | TBRE-Mir 19 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Flex | Extend | Flex | Extend | Flex | Extend | Flex | Extend | Flex | Extend |
| C-0 | -27% | -32% | -39% | -24% | 3% | -1% | -25% | 3% | 2% | 16% |
| C-30 | -31% | -35% | -65% | -65% | NA | NA | -11% | -2% | 16% | -5% |
| C-60 | -35% | -38% | -72% | -69% | NA | NA | -3% | -15% | 18% | -11% |
| C-120 | -43% | -30% | -66% | -65% | -23% | -21% | 12% | 4% | -3% | -13% |
| C-180 | -57% | -48% | -63% | -66% | -24% | -27% | 14% | -11% | 8% | -14% |

The general trend for larger reductions in knee strength at the higher limb velocities suggests that inadequate time for motor unit recruitment or altered recruitment patterns were present in all subjects after space flight. Increasing the volume and intensity of in-flight exercise, modulated the size of the decrease, but did not affect the general trend. Shenkman et al (1997) reported that Mir 19 cosmonauts had little change (-13.8 to 6.7%) in post flight muscle cross-sectional areas (vastus lateralis biopsy), yet these crewmembers still exhibited the same general trend for reductions in strength at higher limb velocities.

## CONCLUSION

We concluded that changes in post flight muscle strength are primarily influenced by alterations in neural control.

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# THE EFFECTS OF A 115-DAY SPACEFLIGHT ON NEUROMUSCULAR FUNCTION IN CREWMAN

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As a result of simulated spaceflight effect (120-day period of bed-rest, 7-day "dry" immersion), contractile properties of the triceps surae muscle (TS) in response to disuse change considerably (Koryak, Eur. J. Appl. Physiol. 1995; Aviat. Space Environ. Med. A60. 1995). We examined maximal twitch response (Pt), maximal voluntary contraction (MVC), maximal force (Po) of isometric contraction elicited by electrical stimulation of tibialis nerve with a supramaximal force and at a frequency of 150 Hz (Koryak, 1978), time-to-peak tension (TPT), a half-relaxation (1/2HR), and time of force development both during voluntary and evoked contractions to 25%, 50%, 75% of maximal before (60-days) and after (6-days) the MIR-18 mission. Force deficit were evaluated as well. TPT increased by 19.1%, 1/2HR by 8.2%, and Pt decreased by 32.9%. Slow and fast MVC, and Po decreased by 35.5%, 26.8%, and 8.2%, respectively. The value Po/Pt ratio increased by 27%. Force deficit increased by 30.3%. The rate of rise a voluntary tension development decreased by 47.8%, 62.5%, and 43.1%, respectively. However, electrical evoked tetanic development not differ substantially from the initial data. These findings indicate relative less functional alterations of the TS compared to tose observed after a 120-days bed-rest that may be related to countermeasure compliance.

# EFFECTS OF 17-DAY SPACEFLIGHT ON HUMAN TRICEPS SURAE ELECTRICALLY-EVOKED CONTRACTIONS

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## INTRODUCTION

Skeletal muscle wasting and weakness have been frequently reported after spaceflight (1). The loss of voluntary muscle strength seems partly explained by atrophy but also by neural adaptations (2). Therefore, the aim of the present study was to investigate the effects of spaceflight on muscle function by-passing changes in neural control through direct electrical stimulation of the muscle.

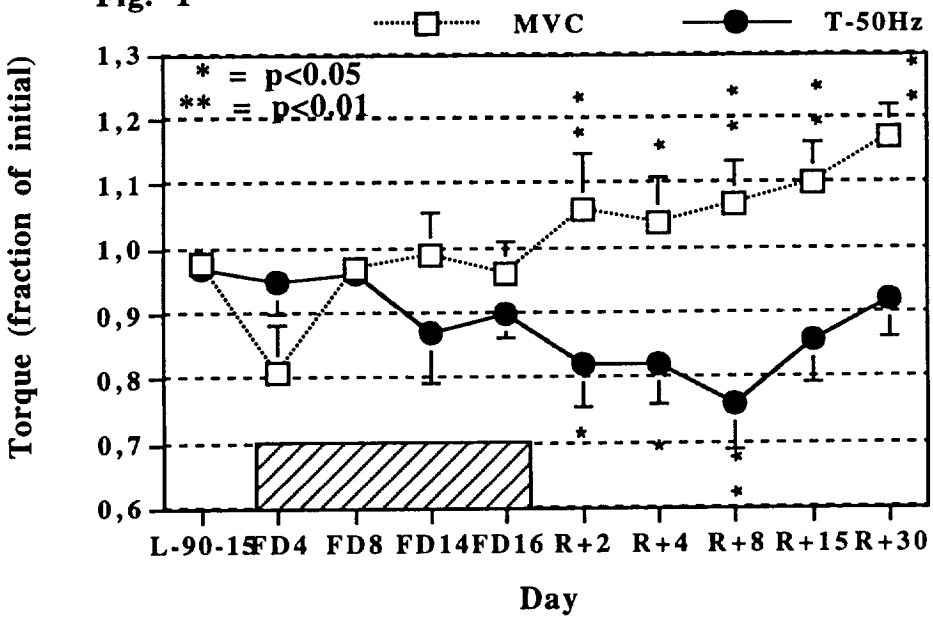
## METHODS

The contractile properties of the human Triceps surae (TS) were investigated on four crew members (age 44.3±3.4 yr., mass 81.0±6.7 kg, height 1.82±0.07 m) of the Life and Microgravity Science (LMS) STS-78 Spacelab mission. Measurements were made before the flight, on days 90, 60, 30, and 15 (L-90, L-60, L-30, L-15), during the flight, on days 4, 8, 14 and 16 (FD4, FD8, FD14, FD16) and during recovery on days 2, 4, 8, 15 and 30 (R+2, R+4, R+8, R+15 and R+30). Time-to peak torque (TPT), half-relaxation time (1/2 RT), rate of rise of torque (dT/dt), and twitch peak torque ($PT_{tw}$) at 20, 15 and 5 deg of dorsiflexion (DF) and at 5, 15, 25 and 30 deg of plantarflexion (PF) were assessed during supramaximal percutaneous electrical stimulation of the Triceps Surae (TS) using the PEMS stimulator (C.I.R., Gals, Switzerland) of the European Space Agency (ESA). Maximum voluntary contraction (MVC) was measured using the twitch-occlusion technique. A current intensity of 60% of the supramaximal level was used to investigate the frequency-torque relation (FTR), during stimulation at 1, 10, 20, 30 and 50 Hz, and the fatiguability during intermittent TS stimulation at 20 Hz with 350 ms trains every second for 2 min. A fatigue index (F.I.) was calculated as the ratio of the final over the initial torque. All torque measurements were performed on the left leg in isometric conditions using the Torque-Velocity-Dynamometer (TVD, E.T.H., Zurich, Switzerland) of ESA. To complement the muscle function data, the muscle plus bone cross-sectional area (CSAm+b) of the calf at mid-tibia was evaluated from anthropometric circumference and skinfolds measurements of the calf.
Values are presented as means±S.E.M.; statistical significance of differences was assessed with repeated ANOVA followed by the Fisher's protected least significance difference test.

## RESULTS

TS tetanic torque at 50 Hz ($T50_{Hz}$) significantly decreased during the flight and recovery, dropping by 24.0±7.0% ($p<0.01$) on R+8 (Fig. 1). Contrary to the loss of tetanic torque, MVC did not change significantly during the flight but progressively increased during recovery reaching a maximum of 17.0%±0.05% on R+30 ($p<0.01$) (Fig. 1). This increase in MVC was accompanied by an improvement in twitch-occlusion in some of the crew members after the flight. The decrease in $T50_{Hz}$ was significantly correlated ($P<0.01$) with a decrease in calf CSAm+b except for values on R+8 at which the loss of torque (24.0±7.0%, $p<0.01$) was far greater than that of CSAm+b (7.1±1.1%, $p<0.01$). On R+15, CSAm+b was still below that of pre-flight by 5.0±1.3% and by R+30 differences were no longer significant. The force/CSA of the TS, calculated by expressing the force at 50 Hz per unit of anatomical CSAm+b, decreased by 16.0±9.5% ($p<0.01$) on R+8. Changes in twitch caracteristics, frequency-torque relation and fatiguability are being presently analysed

Fig. 1




## CONCLUSIONS

The present results show that a significant loss of tetanic torque and cross-sectional area of the Triceps surae occurs during 17 days of spaceflight. It is noteworthy that the tetanic torque continues to decline during the first week of recovery showing a 24% decrease on R+8. This loss of electrically evoked torque was accompanied by a significant decrease in F/CSA which reached its nadir during the reloading phase. A decrease in muscle fibres specific tension (3, 4), muscle damage (5,6), and a change in muscle architecture (5) are proposed as possible explanations for this finding. The maintenance of MVC during spaceflight and an increase during recovery could be due to either: 1) an incomplete motor units activation before the flight and bedrest, supported by the finding of an incomplete twitch-occlusion, 2) a decrease in the co-activation of antagonist muscles, as suggested by EMG recordings of the Tibialis anterior and TS muscles (LMS experiment E-407), and 3) the activation of synergistic and accessory muscles. The present results also suggest that MVC perhaps is not the paradigm of choice for an objective evaluation of muscle deterioration in actual and simulated microgravity.

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# EFFECTS OF MUSCLE UNLOADING ON EMG SPECTRAL PARAMETERS

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## INTRODUCTION

Non-invasive, quantitative techniques are needed to monitor the effects of microgravity on the neuromuscular system. Muscle adaptation to zero gravity and the effectiveness of countermeasures may be assessable by surface electromyographic (EMG) signal techniques [1]. This possibility is primarily based on secondary information, both theoretical and empirical, which have demonstrated that the EMG signal waveform and its propagation velocity are related to muscle fiber cross sectional area (CSA) and metabolite production at the muscle membrane [2]. Changes to these morphological and biochemical properties of muscle have been reported for spaceflight and simulated microgravity [3]. In this study, an in vitro technique was utilized to compare changes in muscle fiber morphology and histochemistry with spectral parameters of the EMG signal in antigravity muscles exposed to short- and long-term unloading.

## METHODS

A tail-suspension procedure [4] was used to induce hindlimb unloading in female Wistar rats, aged approximately 2 months (150-175g). Animals in the experimental group (n=12) were suspended for either seven or twenty-one days. Equal numbers of control animals were included. Neuromuscular preparations of the soleus muscle were extracted at the end of these interventions and placed in an oxygenated isothermal Krebs bath. Preparations were supramaximally stimulated via the nerve at 40 Hz to induce tetanic contractions for a maximum of 30 s. M-waves were recorded using a multielectrode array (O.D. 0.5 mm, interelectrode spacing 2.3 mm) placed against the muscle belly. Signals were sampled at 2048 Hz and then averaged over 0.25 s epochs before calculation of the median frequency by fast fourier transform techniques. The median frequency (MF) is the half-power frequency of the signal spectrum and is used to monitor alterations in the EMG signal that result from changes in the generation and conduction of action potentials at the muscle membrane [2]. We studied two MF parameters: the initial MF, which is the value of MF for the first few epochs of EMG data, and the $\Delta$MF, which is the change in MF between the beginning and end of the 30 s contraction. These parameters are used to describe the EMG signal before and after the effects of fatigue, respectively. Following the experiment, muscles were frozen for later histochemical analysis to measure CSA and fiber type content by myosin ATPase staining. All procedures were conducted following approval by our Institutional Animal Care and Use Committee.

## RESULTS

Tests for significant differences between means were based on a probability of $p<0.05$. Average muscle fiber CSA was significantly decreased when compared to controls at 7 days and 21 days of unloading (Figure 1). There was also a significant increase in the percentage by area of fast fibers, but only for muscles unloaded for 21 days. Initial MF significantly decreased after 7 days of unloading and was further decreased by 21 days of unloading, whereas no significant changes were observed for control muscles (Figure 2). $\Delta$MF was not obtainable for all muscles, and therefore individual data points are shown in this figure rather than grouped data. Unloading did not appear to have an effect on this parameter, although the variability and small sample size preclude a conclusive finding.

## CONCLUSION

The similar pattern of change between the muscle CSA and the initial MF suggests that this EMG parameter is sensitive to the presence of muscle atrophy following muscle unloading. The most likely mechanism for this association is that muscles with a smaller mean CSA have lower muscle fiber conduction velocities which prolong the EMG signal waveform, thereby compressing the spectral content of the signal to lower frequencies. Somewhat unexpectedly, the MF parameters appeared to be insensitive to changes in fiber type because the increase in % fast fibers at 21 days of unloading did not result in an

increase in either initial MF or ΔMF. Such changes have been reported in previous studies comparing normally loaded muscles of different fiber type proportions [5]. It may be that the change in myosin ATPase that occurred following unloading did not involve muscle metabolic factors that could alter the M-wave (e.g. $Na^+$-$K^+$ or $H^+$ ion concentration at the sarcolemma).

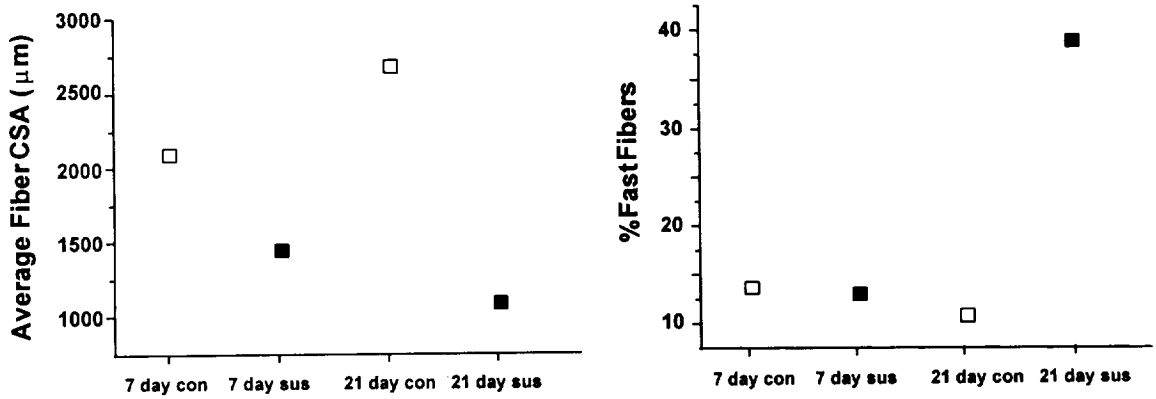


**Figure 1:** Average muscle fiber cross sectional area (CSA) [left plot] and percentage of fast fibers by area [right plot] are shown for control (open squares) and experimental (filled squares) groups at 7 and 21 days. Standard deviation bars are not shown because their magnitude is smaller than the dimensions of the data point symbols.

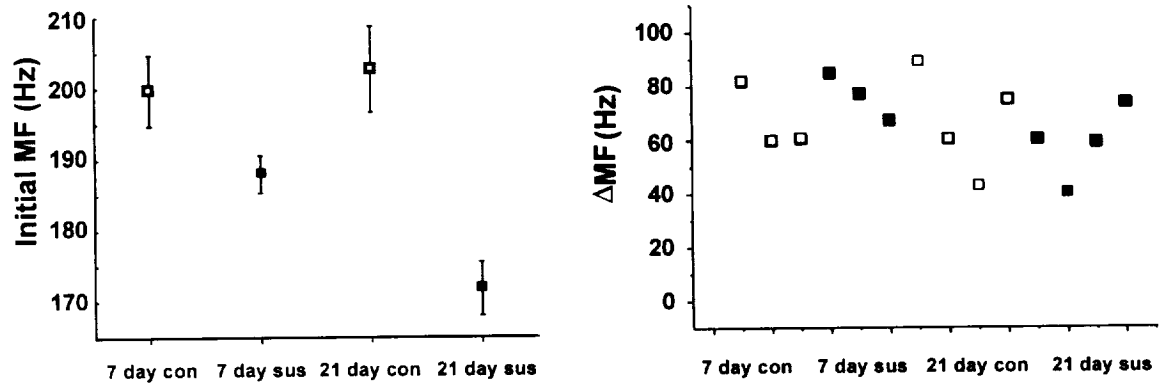


**Figure 2:** Mean (SD bars) of the initial MF for M-waves elicited from control muscles (open squares) and unloaded muscles (filled squares) at 7 and 21 days [left plot]. Change in MF (ΔMF) following the 30 s contraction for several samples of control (open squares) and unloaded (filled squares) muscles at 7 and 21 days [right plot].

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# MYOFIBER WOUND-MEDIATED FGF RELEASE AND MUSCLE ATROPHY DURING BEDREST.

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## INTRODUCTION

We have previously shown that there is a linear relationship between the amount of mechanical load placed on muscle tissue and the amount of myofiber fibroblast growth factor (FGF) (acidic and basic isoforms) released into the extracellular environment both *in vivo* and *in vitro*. In addition. we have demonstrated *in vitro* that the growth-promoting effect of mechanical load upon human skeletal myotube cultures (analogous to myofibers *in vivo*) can be specifically inhibited by the presence of a site-directed anti-FGF antibody in the tissue culture medium. We postulate that a reduction in mechanical load-induced myofiber wounding and a consequent decrease in wound-mediated release of FGF under microgravity conditions contributes to the initiation of skeletal muscle atrophy during spaceflight. Our hypothesis is supported by experimental data gathered from Space Shuttle crew-members which indicates that circulating levels of a skeletal myofiber-specific wound marker, the MM isoform of creatine kinase (CKMM), is significantly reduced after short duration spaceflight. We have tested our hypothesis utilizing a terrestrial model of human adaptation to spaceflight (i.e. 14 days of 6° degree head-down tilt bedrest) and determined the amount of myofiber wounding and FGF release which occurs during mechanical unloading of the human body. In addition, a resistive exercise protocol was incorporated into the experimental design in order to test the efficacy of resistive exercise as a countermeasure to skeletal muscle atrophy induced by mechanical unloading.

## METHODS

The level of myofiber wounding in bedrest subjects with, and without, resistive exercise was assessed by determining the amount of the CKMM iosform present in the serum before and after 14 days of 6° head-down tilt bedrest. Total CK activity was determined using a commercially available assay for CK based upon the conversion of NADP to NADPH (Roche Diagnostic Systems, Inc., NJ) measured at 340 nm using a Cobas Mira Chemical Analyzer (Roche Diagnostic Systems, Inc., NJ). In addition, CK isoenzyme profiles were also determined from the same samples using the commercially available automated Paragon Gel System (Beckman, Fullerton, CA). This system separates CK isoforms using agarose gel electrophoresis followed by incubation of the gel in CK substrate buffer containing creatine phosphate, hexokinase, ATP, D-glucose, glucose-6-phosphate and NADP as the major components, followed by fluorescent densitometry as described by the manufacturer. Serum levels of both acidic and basic FGF were also determined before and after bedrest using a previously described ELISA protocol developed in this laboratory (Clarke and Feeback, 1996: FASEB J.10: 502-509). In addition, a needle biopsy was performed on the same region of the *m. vastus lateralis* of each subject before and after bedrest. Muscle samples were snap-frozen in liquid nitrogen-cooled iospentane, cryosectioned and myofiber cross-sectional area was determined using quantitative image analysis. A small amount of each muscle biopsy sample (i.e. 50 μg) was homogenized and the amount of FGF present in the soluble protein fraction was determined by ELISA. A third experimental group underwent resistive exercise but was not bedrested. These ambulatory, resistive exercise-trained subjects were used as positive controls for mechanical load-induced myofiber wounding but did not undergo the muscle biopsy procedure.

## RESULTS

Bedrest alone caused a significant ($p < 0.05$; n=8) decrease in myofiber size of the *m. vastus lateralis* (**Figure 1**). Muscle atrophy was paralleled by significant ($p < 0.05$; n=8) reductions in the serum levels of both CKMM (**Figure 2**) and aFGF (**Figure 3**). In contrast, bedrest plus resistive exercise resulted in the reversal of unloading-induced skeletal muscle atrophy (**Figure 1**). This reversal was paralleled by significant ($p < 0.05$;n=8) increases in the serum levels of both CKMM (**Figure 2**) and aFGF (**Figure 3**). In addition, exercise-induced reversal of myofiber atrophy in the *m. vastus lateralis* was paralleled by a significant ($p < 0.05$; n=8) increase in the amount of myofiber-associated aFGF (**Figure 4**) detected in the soluble protein fraction of homogenized muscle biopsy material. No significant changes were detected in serum or myofiber-associated bFGF levels in the three experimental groups (data not shown). As expected, resistive exercise in ambulatory subjects resulted in a significant ($p < 0.05$; n=6) increase (i.e. a 50% increase) in serum CKMM levels (**Figure 2**), although no significant increase in the amount of FGF released into the serum was noted in these subjects (**Figure 3**). However, bedrest plus resistive exercise caused significantly ($p < 0.05$; n=8)) greater amounts of CKMM to be released into the serum (i.e. a 450% increase) than would be predicted based on the levels released in the ambulatory exercised group (**Figure 2**).

**Figure 1**

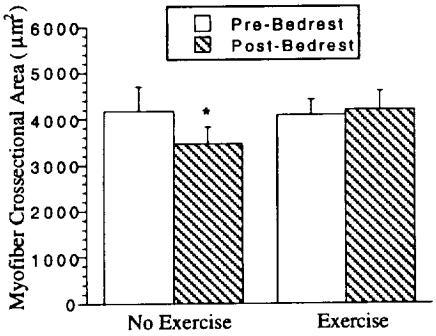



**Figure 2**

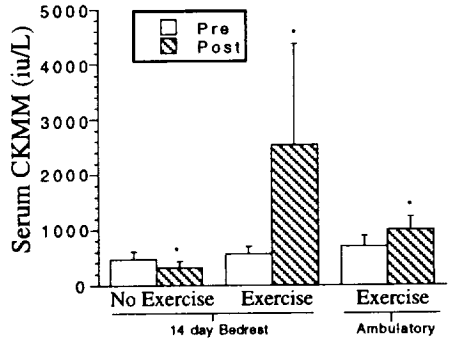



**Figure 3**

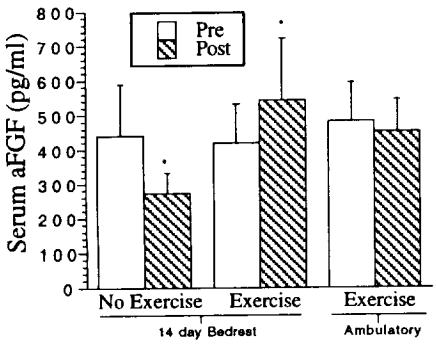
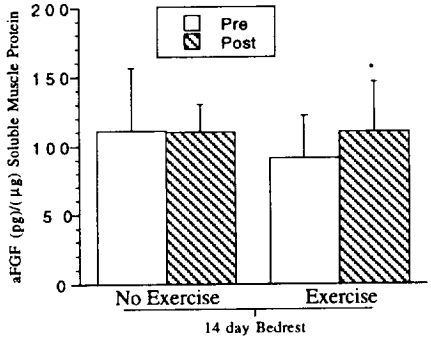



**Figure 4**




CONCLUSIONS

Our experimental results in a bedrest model of microgravity-induced skeletal muscle atrophy indicate that a reduction in mechanical-induced myofiber wounding and disruption of wound-mediated FGF release from the myofiber cytoplasm plays a central role in the initiation of muscle atrophy. Our results indicate that mechanical unloading inhibits FGF release, rather than FGF content, in skeletal muscle tissue. Whether or not FGF is the only growth factor involved in skeletal muscle atrophy remains unclear. However, sarcolemmal wounding and consequent membrane wound-mediated FGF release is a mechanically-reactive signaling pathway which can be directly linked to a muscle growth response. As such, it is a leading candidate for potential disruption during spaceflight. In addition, FGF may have a series of direct and indirect effects upon several other components of skeletal muscle tissue, all of which play a role in the maintenance of skeletal muscle mass. For example, FGF is capable of modulating the function of other muscle growth factors, such as insulin-like growth factor-1 (IGF-1) where FGF upregulates the expression of IGF-1 protein and its receptors in human muscle cells. A second example of the importance of FGF is its role in the maintenance of neuronal cell function and integrity, where FGF is responsible for the upregulation of nerve growth factor (NGF) protein and its receptors. In addition, FGF is a potent angiogenic factor for microvascular endothelial cells within the muscle capillary bed.

Our experimental observations in bedrest plus resistive exercise subjects indicate that skeletal myofibers are more prone to mechanical load-induced sarcolemmal damage after a period of unloading than ambulatory, resistive exercised individuals. These data suggest that not only is there a myofibrilar remodeling component involved in the adaptive muscle response to mechanical unloading, but that there is an additional membrane remodeling component. We are now in the process of analyzing the lipid composition of sarcolemma membranes isolated from bedrest muscle biopsy material to determine if this is the case. It is still unclear whether membrane remodeling occurs before or after myofibrilar protein remodeling, or whether both occur at the same time during the adaptive response.

In conclusion, is interesting to speculate that a microgravity-induced reduction in mechanically-induced, membrane wound-mediated FGF release from skeletal myofibers may lead not only to myofiber atrophy, but that disruption of this signaling pathway may also play a role in the disruption of other signaling pathways involved in the maintenance of muscle mass, such as neuromuscular function and maintenance of muscle capillarity.