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EFFECTS OF UNLOADING AND RESISTANCE EXERCISE ON SKELETAL MUSCLE FUNCTION, SIZE AND COMPOSITION IN MAN

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ABSTRACT

Exposure to microgravity, i.e., spaceflight, causes muscle unloading leading to muscle atrophy and dysfunction. Thus, there is a need for effective countermeasures to combat these effects. The present thesis aimed to study function, size and composition of anti-gravity muscles following long-duration bed rest, a valid spaceflight analogue. A further and even more important aim was to study the effects of concurrent resistance exercise using a gravity-independent device.

Prior to this, the exercise paradigm was validated during space station-like conditions. Four healthy men trained 2-3 times weekly during 110 days of confinement in a ground-based chamber, severely restricting locomotor activity. Training performance progressed over time and maximal voluntary contraction (MVC) was either increased or maintained after confinement. Since the device showed feasibility and potential as a countermeasure against muscle function deterioration, it was subsequently employed during strict unloading.

Nine healthy men performed 90 days of bed rest (BR), while another group of eight men in addition carried out resistance exercise for the knee extensors and plantar flexors every third day (BRE). Different indices of muscle function were obtained together with surface electromyographic (EMG) amplitude before and after the intervention. Muscle volume was assessed by means of magnetic resonance imaging (MRI) prior to and on day 29 and 89 during bed rest. Moreover, muscle biopsies were obtained from mm. vastus lateralis (VL; all subjects) and soleus (n=3 from each group) before and on day 84 during bed rest, for subsequent analyses of single fibre myosin heavy chain (MHC) content.

In BR, muscle volume of the knee extensors decreased ($p < 0.05$) by 10 and 18% on day 29 and 89, respectively. The corresponding decreases for the plantar flexors were 16 and 29%, respectively. In BRE, knee extensor atrophy was prevented ($p > 0.05$), while the more pronounced plantar flexor atrophy was attenuated (-8 and -15%). Maximal torque, force and power, measured during different types of actions, decreased by 31-60% in BR. In BRE, MVC was maintained for the knee extensors but not for the plantar flexors. Training-specific force and power were unaltered for both muscles, while maximal torque measured in actions different from the training task, decreased. EMG amplitude decreased during maximal and increased during submaximal actions in BR, but not in BRE. BR, but not BRE, showed increased fatigability and decreased rate of force development (RFD). In BR, there was an increase in hybrid fibres and a shift towards faster phenotypes in both VL and soleus. In BRE, this effect was attenuated in VL and offset in soleus. The phenotype shift was not manifested in altered force-velocity characteristics.

The greater atrophy of the plantar flexors compared to the knee extensors in response to unloading, may be explained by the greater content of slow fibres and the more frequent use of this particular muscle group in daily life. Further, muscle volume and single fibre data suggest that slow fibres are less responsive to the training protocol. The present findings also provide evidence that neural mechanisms, in addition to changes in muscle size, contribute to muscle function alterations induced by bed rest with or without resistance exercise, while phenotype shift may play a more modest role. Hence, it is clear that designing countermeasures for in-flight use extends beyond preserving muscle size only. Though the present work was spurred by questions addressed through the human spaceflight program, the results do have important clinical implications for e.g., aging populations or patients undergoing atrophy due to disease or injury.

Key words: *atrophy, bed rest, confinement, countermeasure, electromyography, human, knee extensors, magnetic resonance imaging, microgravity, muscle function, myosin heavy chains, plantar flexors, resistance exercise, skeletal muscle, spaceflight, strength, training specificity, unloading*

POPULÄRVETENSKAPLIG SAMMANFATTNING

Människan har genom evolutionen skapats för regelbundet muskelarbete. Om musklerna inte får arbeta förtvinar de och deras funktion försämras. Detta sker vid skada och sjukdom, men även till följd av en stillasittande och inaktiv livsföring. Liknande förändringar ses hos astronauter som vistas i tyngdlöshet. De drabbar framför allt de muskler som arbetar mot gravitationen och håller oss upprätta, till exempel vad- och knästräckarmuskulatur.

Vetenskapliga studier av astronauter är dock svåra att genomföra. Det finns få försökspersoner och det är svårt att kontrollera studierna. För att simulera effekterna av tyngdlöshet används därför olika avlastningsmodeller, till exempel sängvila och avlastning av ena benet med hjälp av kryckor. Sådana avlastningsstudier har bekräftat de knapphändiga resultat som finns från studier av astronauter. De flesta av dessa har dock varat kortare tid än sex veckor och längre studier är därför önskvärda.

Det är angeläget att hitta sätt att motverka de negativa effekter som vistelse i tyngdlöshet leder till. Styrketräning ger muskelförstoring och styrkeförbättring och har vid kortare avlastningsstudier visat sig förebygga muskelförtvinning och styrkenedgång. Vid avsaknad av gravitation fungerar dock inte traditionell styrketräning, och därmed måste andra metoder för att åstadkomma motstånd användas. Tidigare använda träningsredskap har inte varit tillräckligt effektiva. Därför har en styrketräningsapparat som utnyttjar tröghetsmomentet hos roterande svänghjul, en så kallad svänghjulsergometer, konstruerats för gravitationsoberoende styrketräning. Målsättningen med denna avhandling var att studera effekten av långvarig avlastning på skelettmuskulaturens funktion, storlek och sammansättning samt hur styrketräning med en svänghjulsergometer kan motverka de förväntade negativa följderna.

I ett första steg utvärderades om en svänghjulsergometer kan användas under rymdstationsliknande förhållanden och hur effektiv den är. Fyra män var instängda i en kammare i 110 dagar. Även om denna modell inte ger total avlastning, begränsas muskelaktiviteten kraftigt. Två till tre dagar i veckan styrketränade testpersonerna med en svänghjulsergometer. Sex olika övningar för alla de viktigaste musklerna – däribland vader, knästräckare och ryggmuskulatur – ingick i programmet. Det utförda arbetet mättes under varje träningspass. Försökspersonerna tränade själva och kunde se på en datorskärm vad de presterade. Före och efter försöksperioden genomfördes mätningar av muskelstyrkan. Försökspersonerna kunde öka belastningen under perioden och styrkan i de muskler som tränades ökade eller var oförändrad. Träning med svänghjulsergometern var således effektiv och kunde praktiskt genomföras under rymdstationsliknande förhållanden.

I nästa steg studerades effekten av total muskelavlastning med eller utan styrketräning. Sjutton män låg till sängs i 90 dagar och fick inte lämna den liggande positionen någon gång under studien. Medan nio personer enbart låg till sängs, utförde åtta personer styrketräning för vader och knästräckare var tredje dag. Styrketräningen genomfördes liggande på en bädd utrustad med en modifierad svänghjulsergometer. Före och efter sängvilan mättes olika typer av muskelstyrka. Muskelaktiviteten mättes med elektromyografi (EMG), med hjälp av elektroder på huden över respektive muskel. Muskelstorleken mättes med en magnetkamera innan försöket inleddes samt dag 29 och 89 under sängvilan. Muskelprover för vidare analys av muskelfibertyper togs före och dag 84 under sängvilan.

Hos de testpersoner som inte styrketränade minskade knästräckarnas muskelvolym med 10 procent till dag 29 och 18 procent till dag 89. Motsvarande siffror för vadera var 16 respektive 29 procent. Vadmusklerna förtvinade alltså mer än knästräckarna. Detta skulle kunna förklaras av att vadmusklerna används mer vid daglig aktivitet som

stående och gående, varför avsaknaden av sådana aktiviteter kan tänkas bli mer kännbar för vaderna. Vidare innehåller vaderna en större andel långsamma muskelfibrer. Större förtvinning av långsamma än av snabba muskler har tidigare visats hos smågnagare. Den här studien visar tydligt att detta gäller även för människor, vilket har varit tvetydigt i tidigare studier.

Styrketräning motverkade helt förtvinningen av knästräckarna. Det är värt att notera att tiden för träning endast motsvarade 0,03 procent av tiden i sängvila. Detta räckte uppenbarligen för att bibehålla knästräckarnas muskelvolym. Däremot bevarades inte vadernas muskelvolym helt av styrketräningen. Träningsgruppen uppvisade en förtvinning på 8 procent dag 29 och 15 procent dag 89. Den större förtvinningen hos vaderna gör att det blir svårare att bibehålla muskelvolymen med styrketräning. Tidigare studier har visat att ett visst styrketräningsprogram ger sämre resultat för vadmuskler och långsamma muskelfibrer.

Olika mått på maximal styrka visade en nedgång på 31–60 procent. Styrkan minskade således mer än muskelvolymen. Denna skillnad tyder på förändringar i nervsystemet och/eller andra muskulära förändringar som är avgörande för förmågan att utveckla kraft. Muskelaktiviteten mätt med EMG minskade vid maximal ansträngning, vilket tyder på att förmågan att aktivera musklerna när de arbetar maximalt minskar efter avlastning. Vidare ökade EMG-aktiviteten vid arbete på en låg given belastning, vilket tyder på att en större del av muskeln måste användas för att utföra ett visst arbete. Detta kan bidra till att muskeln lättare blir uttröttad, vilket även visade sig genom en mer markant förändring av EMG-signalen över tid vid ihållande muskelarbete.

Efter sängvilan hade andelen muskelfibrer av snabb karaktär ökat i både yttre knästräckarmuskeln (m. vastus lateralis) och djupa vadmuskeln (m. soleus), något som inte har kunnat påvisas vid kortare studier. Denna förändring var mindre hos träningsgruppen. Skiftet mot mer snabba fibrer kunde förväntas påverka det så kallade kraft-hastighets sambandet, vilket innebär att nedgången i muskelstyrka är mindre tydlig vid högre hastigheter. Förmågan till snabb kraftutveckling kunde också förväntas bli bättre. Några sådana förändringar sågs dock inte i denna studie. Det tyder på att de muskelfibertypsförändringar som sker vid avlastning inte påverkar muskelfunktionen på ett avgörande sätt.

Parallellt med förändringen i muskelstorlek resulterade styrketräningen i bibehållen maximal statisk styrka för knästräckarna men inte för vaderna. Styrka som tränats i övningarna bevarades emellertid för båda muskelgrupperna. Detta tyder på att även andra anpassningar skedde i vadmuskulaturen. Förmågan att snabbt utveckla kraft och att genomföra en uttröttande övning var också oförändrad i träningsgruppen. Däremot hade styrketräningen mycket mindre effekt på både vad- och knästräckarmuskulatur när styrkan mättes i övningar som avsevärt skiljde sig från träningen, även om det var samma muskler som ansträngdes. Detta visar att bevarande av muskelmassan inte nödvändigtvis leder till bibehållen muskelstyrka.

Sammanfattningsvis visar denna avhandling att vadmuskulaturen förtvinar mer än knästräckarmuskulaturen vid långvarig sängvila. Muskelförtvinning motverkades för knästräckarna och mildrades för vaderna med styrketräning utförd var tredje dag. Fibertyp-fördelningen förändrades efter avlastning och denna effekt var mindre i träningsgruppen. Dessa förändringar hade dock ingen större betydelse för musklernas funktion. Funktionen bibehölls bäst i tränings-specifika övningar, vilket visar på betydelsen av funktionella tränings- och testprogram. Dessa resultat är betydelsefulla för utvecklandet av träningsprogram för astronauter men också för andra grupper, till exempel äldre och patienter som drabbas av muskelförtvinning som en följd av sjukdom eller skada.

Look at a patient lying long in bed.
What a pathetic picture he makes!
The blood clotting in his veins,
the lime draining from his bones,
the scybala stacking up in his colon,
the flesh rotting from his seat,
the urine leaking from his distended bladder,
and the spirit evaporating from his soul.

(From "The dangers of going to bed" by R. A. J. Asher, British Medical Journal, 1947)

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LIST OF ABBREVIATIONS

BR	Subject group performing bed rest only
BRE	Subject group performing bed rest and resistance exercise
Con	Concentric
CV	Coefficient of variation
Ecc	Eccentric
EMG	Electromyography
FW	Flywheel
GL	Gastrocnemius lateralis
GM	Gastrocnemius medialis
MHC	Myosin heavy chain
MRI	Magnetic resonance imaging
MVC	Maximal voluntary contraction (Isometric)
RF	Rectus femoris
RFD	Rate of force development
RMS	Root mean square
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
VL	Vastus lateralis
VM	Vastus medialis

INTRODUCTION

The human body has evolved to perform weight-bearing activities. When this stimulus is reduced or absent e.g., due to disease, injury, a sedentary lifestyle or aging, anti-gravity muscle function deteriorates (Adams *et al.* 2003, Asher 1947). Perhaps the most striking challenge in this regard, is the lack of gravity experienced by astronauts or cosmonauts during spaceflight that ultimately could jeopardize crew health and performance and hence also mission completion (cf. Greenleaf *et al.* 1989, Nicogossian *et al.* 1994b). Thus, to assure safe and successful spaceflights, countermeasures to this effect are urgently warranted (Kozlovskaya and Egorov 2003).

However, this calls for more thorough studies regarding the effects of spaceflight on skeletal muscle function, size and integrity as well as studies establishing the efficacy of potential countermeasures, e.g., physical exercise, “artificial gravity”, nutritional or pharmacological interventions. Unfortunately, flight opportunities and access to space travellers to be examined under strictly controlled conditions and allowing for interventions to be employed, are very limited, if available at all. Hence, ground-based spaceflight analogues are necessary and currently used, in efforts to generate data that would aid in understanding how human skeletal muscle adapts to chronic exposure to unloading mirroring microgravity conditions. Models that have been proven valid and feasible include horizontal or head-down bed rest (Berg *et al.* 1997, Deitrick *et al.* 1948, Fortney *et al.* 1996, Taylor *et al.* 1945), dry-water immersion (Koryak 1995b, 1998b, 2002) and unilateral lower-limb unloading (Berg *et al.* 1991, Dudley *et al.* 1992, Hather *et al.* 1992, Schulze *et al.* 2002, Tesch *et al.* 2004b). Confinement in a ground-based chamber, resembling a space station (Bonting 1993), does not cause complete muscle unloading, but will certainly minimize locomotor activity (Vaernes *et al.* 1993).

Few studies investigating skeletal muscle responses to unloading have exceeded six weeks (Koryak 1995a, 1998a, LeBlanc *et al.* 1992, Ohira *et al.* 1999, Shackelford *et al.* 2004) and results are far from conclusive. Since tentative future spaceflights may include missions to Mars or other planets, and extend beyond several months or even years, further research disclosing how muscle function is modified due to long-duration unloading and impacted by countermeasures, are warranted.

EFFECTS OF REAL AND SIMULATED SPACEFLIGHT ON MUSCLE

The sparse and sometimes sketchy data generated following spaceflight of varying duration, collectively shows that muscle function impairment is marked (Adams *et al.* 2003, Antonutto *et al.* 1999, Greenleaf *et al.* 1989, Koryak 2001, Lambertz *et al.* 2003, Lambertz *et al.* 2001, Rummel *et al.* 1975, Tesch *et al.* 2005). The very obvious decrease in maximal voluntary strength upon return to Earth appears to be attributed to muscle atrophy mainly (Akima *et al.* 2000a, Edgerton *et al.* 1995, LeBlanc *et al.* 2000a, LeBlanc *et al.* 1995, Zange *et al.* 1997), and due to altered muscle protein metabolism (Ferrando *et al.* 1996, Stein *et al.* 1999). However, given the greater relative decay in maximal strength than size demonstrated following real or simulated spaceflight (Adams *et al.* 1994, Akima *et al.* 2001, Berg *et al.* 1991, Berg *et al.* 1997, LeBlanc *et al.* 1992, Ploutz-Snyder *et al.* 1995, Schulze *et al.* 2002, Tesch *et al.* 2004b, Zange *et al.* 1997), it is generally agreed that atrophy alone cannot account for the strength loss. Hence, neuronal adaptations (Antonutto *et al.* 1999, Berg *et al.* 1997, Duchateau 1995,

Dudley *et al.* 1992, Koryak 1995a, 1996, Koryak 2001, Ruegg *et al.* 2003) and alterations in the contractile machinery, e.g. compromised cross bridge interaction (Fitts *et al.* 2000, 2001, Larsson *et al.* 1996, Widrick *et al.* 1999), may add to this effect.

Controlled studies using spaceflight analogues of up to 6 weeks duration, have shown weekly decreases in maximal strength of the postural knee extensor and plantar flexor muscles of about 4-5% (Adams *et al.* 1994, Akima *et al.* 2001, Bamman *et al.* 1998, Bamman *et al.* 1997, Berg *et al.* 1991, Berg *et al.* 1997, Berg and Tesch 1996, Dudley *et al.* 1992, Dudley *et al.* 1989, Germain *et al.* 1995, Gogia *et al.* 1988, Ploutz-Snyder *et al.* 1995, Schulze *et al.* 2002, Tesch *et al.* 2004b) and a somewhat smaller rate of muscle atrophy corresponding to about 2-3 % (Adams *et al.* 1994, Akima *et al.* 2001, Akima *et al.* 2003, Berg *et al.* 1991, Berg *et al.* 1997, Berry *et al.* 1993, Convertino *et al.* 1989, Ferrando *et al.* 1995, Hather *et al.* 1992, LeBlanc *et al.* 1988, Ploutz-Snyder *et al.* 1995, Schulze *et al.* 2002, Tesch *et al.* 2004b). The very limited data at hand following more prolonged unloading, infer that the rates of muscle and strength loss, level off within the first 120 days of unloading (Koryak 1999a, LeBlanc *et al.* 1997, LeBlanc *et al.* 1992, Shackelford *et al.* 2004).

It is evident that strength loss and atrophy of the postural muscles are significant and prominent (Adams *et al.* 1994, Hather *et al.* 1992, LeBlanc *et al.* 1988). However, the greater atrophy of the “slow” soleus compared with “faster” muscles that is manifest following unloading and spaceflight in lower mammals (cf. Fitts *et al.* 2000, Thomason and Booth 1990), has not been confirmed in controlled human studies. Whereas some studies show more pronounced atrophy of the plantar flexor muscles compared with the knee extensors (Akima *et al.* 2001, Hather *et al.* 1992, LeBlanc *et al.* 1992), other reports show no difference (Berry *et al.* 1993, Schulze *et al.* 2002, Tesch *et al.* 2004b) or even greater global knee extensor muscle atrophy (Convertino *et al.* 1989). In lower mammals, slow (type I) fibres show greater atrophy than fast (type II) fibres (cf. Fitts *et al.* 2000, Talmadge 2000, Thomason and Booth 1990). Though such a response appears evident in both the soleus and vastus lateralis (VL) muscles in patients following trauma (Edström 1970, Häggmark and Eriksson 1979, Häggmark *et al.* 1981), no selective fibre atrophy of the VL muscle was shown in healthy subjects after 16-30 days unloading (Adams *et al.* 1994, Berg *et al.* 1993a, Hikida *et al.* 1989). In fact and somewhat surprisingly, in astronauts returning to Earth from short-duration missions in Orbit, investigators even reported greater type II than type I fibre atrophy (Edgerton *et al.* 1995, Widrick *et al.* 1999).

The muscle fibre contractile properties are in part controlled by phenotype, i.e., myosin heavy chain (MHC) composition (Bottinelli 2001, Bottinelli *et al.* 1996, Larsson and Moss 1993, Widrick *et al.* 1996). A transition from slower to faster muscle fibre types has been evident following hind-limb suspension and spaceflight in lower mammals (Fitts *et al.* 2000, Ohira *et al.* 1992, Oishi *et al.* 1998, Talmadge 2000, Thomason and Booth 1990). In humans, data from spaceflight (Widrick *et al.* 1999, Zhou *et al.* 1995) and immobilization following trauma (Häggmark *et al.* 1986) showed a similar shift. However, muscle unloading of healthy subjects did not provoke a change in fibre type composition of the human VL or soleus muscles (Adams *et al.* 1994, Bamman *et al.* 1998, Berg *et al.* 1993a, Berg *et al.* 1997, Ferretti *et al.* 1997, Hikida *et al.* 1989). These interventions were shorter than 37 days and it has been inferred that more extended unloading may provoke a phenotype shift; an

ongoing transition at the mRNA level was demonstrated following 37 days of bed rest (Andersen *et al.* 1999), and there was a tendency towards a slow-to-fast phenotype shift after four months of bed rest (Ohira *et al.* 1999).

A shift towards faster phenotypes would potentially impact *in-vivo* muscle function. Differences in e.g., altered force-velocity curve such that force would increase at higher velocities, increased rate of force development (RFD) and augmented fatigue susceptibility, have been seen in subjects with different phenotype profile (Aagaard and Andersen 1998, Gür *et al.* 2003, Harridge *et al.* 1996, Thorstensson *et al.* 1976a). Associated changes in MHC composition and *in vivo* force-velocity relationship were also evident following detraining subsequent to chronic resistance exercise (Andersen *et al.* 2005). Thus it may be that simulated long-term spaceflight is associated with altered *in-vivo* muscle function as suggested above.

Previous observations suggest that motor control is modified following unloading or spaceflight. For example, there was an increase in muscle use, as reflected in increased surface EMG amplitude or contrast shift of Magnetic Resonance Images (MRI), in response to a given exercise task following real or simulated spaceflight (Berg and Tesch 1996, Kozlovskaya *et al.* 1981, Ploutz-Snyder *et al.* 1995). Moreover, chronic absence of weight-bearing provoked increased fatigability (Portero *et al.* 1996) whereas RFD was unaltered or decreased (Bamman *et al.* 1998, Koryak 1995a, 1998a, b, Koryak 1998c, Kubo *et al.* 2000). Combined, these findings would suggest that motor control during submaximal or maximal tasks may be altered as a consequence of unloading.

EFFECTS OF RESISTANCE EXERCISE

Since long, resistance exercise, i.e., high force - low repetition actions, has been known to increase maximal voluntary strength (De Lorme *et al.* 1952). It is generally held that this effect initially is mediated mainly by neural factors, while muscle hypertrophy may play a more significant role several weeks into a training programme (Häkkinen 1989, Moritani and deVries 1979, Narici *et al.* 1989, Sale 2003). The performance of maximal or near maximal eccentric actions during resistance training appears crucial to optimize muscle hypertrophy (Dudley *et al.* 1991, Hather *et al.* 1991), and may also play a central role in increasing neural drive, i.e. by bringing more motor units into action and/or increasing firing rate of individual motor units (Aagaard 2004, Enoka 1997, Sale 2003).

The complexity of neural adaptations taking place in response to chronic resistance exercise is readily apparent from numerous reports that have demonstrated modest increases in non training-specific tasks despite robust gains in training specific performance, emphasizing the same muscle or muscle group (e.g. Enoka 1997, Morrissey *et al.* 1995, Rasch and Morehouse 1957). Thus depending on exercise and testing mode, marked differences with regard to strength increases for e.g., unilateral vs. bilateral (Häkkinen *et al.* 1996, Taniguchi 1997), isotonic vs. “isokinetic” (Sleivert *et al.* 1995, Wilson and Murphy 1995), single vs. multi-joint (Sale *et al.* 1992), concentric vs. eccentric vs. isometric (Hortobágyi *et al.* 1997) actions, and for actions measured at different joint angles (Lindh 1979), have been shown. Resistance exercise enhances RFD, and particularly by programmes including more explosive actions

(Aagaard *et al.* 2002, Häkkinen and Komi 1986, Thorstensson *et al.* 1976b, Van Cutsem *et al.* 1998). There is also evidence that resistance exercise is capable of promoting a phenotype change, i.e., a decrease in hybrid fibres (Williamson *et al.* 2001) and an increase in the total amount of MHC IIa at the expense of MHC IIx (cf. Tesch and Alkner 2003). Altogether, physiological adaptations of skeletal muscle, manifest following chronic resistance exercise, would suggest that the changes associated with muscle dysfunction caused by unloading could be counteracted in part or fully by resistance exercise.

RESISTANCE EXERCISE COUNTERMEASURE DURING UNLOADING

Indeed, previous short-term (five weeks or less) space-simulation studies have shown that resistance exercise could prevent muscle atrophy (Akima *et al.* 2001, Akima *et al.* 2000b, Bamman *et al.* 1998, Bamman *et al.* 1997, Schulze *et al.* 2002, Tesch *et al.* 2004b) and decreases in maximal voluntary strength (Akima *et al.* 2001, Akima *et al.* 2000b, Bamman *et al.* 1998, Germain *et al.* 1995, Schulze *et al.* 2002, Tesch *et al.* 2004b) of the knee extensor muscles. While some short-term studies have reported maintained plantar flexor muscle size and strength when resistance exercise was carried out during bed rest (Akima *et al.* 2003, Bamman *et al.* 1997), other studies failed to offset these effects (Akima *et al.* 2001, Akima *et al.* 2000b, Shackelford *et al.* 2004).

Furthermore, the principle of specificity has been obvious also when using resistance exercise as a countermeasure during unloading. Whilst performance during training specific tasks and RFD were maintained, other non training-specific tasks involving the knee extensors were not (Bamman and Caruso 2000, Bamman *et al.* 1998).

The results of studies which have determined potential muscle phenotype changes following unloading with or without exercise countermeasures are inconclusive (Bamman *et al.* 1998, Ohira *et al.* 1999). In light of previous observations in rodents (cf. Edgerton and Roy 1995) and after unloading and resistance exercise respectively, this issue certainly needs to be further investigated. Collectively, there is only fragmental data available (Koryak 1998a, Shackelford *et al.* 2004), examining in detail, various aspects of skeletal muscle adaptations in individuals subjected to long-duration unloading with and without resistance exercise.

TRAINING IN SPACE

For obvious reasons and given the well established physiological adaptations that occur in response to chronic resistance exercise, this paradigm has been proposed as a countermeasure during spaceflight (Baldwin *et al.* 1996, Convertino 2002, Nicogossian *et al.* 1994a). However, performing resistance exercise in the microgravity environment is challenging since standard weights cannot be used. Thus, alternative means of producing resistance and simulating weight lifting must be at hand in space. Previous training programs, the vast majority emphasizing low force and concentric actions, employed during spaceflight (Lee *et al.* 2004, Nicogossian *et al.* 1994a), failed to counteract muscle atrophy and strength loss (Akima *et al.* 2000a, Antonutto *et al.* 1999, Edgerton *et al.* 1995, Lambertz *et al.* 2003, Lambertz *et al.* 2001, LeBlanc *et al.* 2000a, LeBlanc *et al.* 1995, Rummel *et al.* 1975, Zange *et al.* 1997). To meet the needs of an effective resistance exercise device for astronauts, a gravity-independent resistive

exercise device using the inertia of spinning flywheels to allow for coupled concentric and eccentric forces, has been developed (Berg and Tesch 1994, 1998). Eccentric forces are performed during daily locomotor activities on earth but are rarely performed in microgravity. Thus, they should be included in countermeasure programs in space (Hargens *et al.* 1989). Use of this exercise apparatus has been proven effective in inducing hypertrophy and increased muscle strength at 1g (Tesch *et al.* 2004a) and, more importantly, men and women subjected to five weeks of lower limb unloading and performing knee extension exercises 2-3 times per week, showed maintained maximal strength and even increased muscle volume (Tesch *et al.* 2004b). These data are promising and indicate that this particular resistance exercise paradigm could serve as an important aid to maintain normal skeletal muscle physiology on long-duration missions in space.

AIMS

The general aim of this thesis was to study the effects of long-term unloading with or without concurrent resistance exercise on lower limb skeletal muscle size, function and composition. More specifically the aims were to study:

- feasibility and efficacy of a gravity-independent resistance exercise device employed during long-term confinement (paper I)
- validity and accuracy of this ergometer for functional testing and training of bedridden individuals (paper II)
- knee extensor and plantar flexor atrophy following short- and long-term muscle unloading, and compare responses of these two muscle groups (paper II and III)
- changes in different indices of knee extensor and plantar flexor muscle function following long-term unloading (paper III)
- the effects of resistance exercise, on knee extensor and plantar flexor muscle volume following short- and long-term unloading (paper II and III)
- the effects of resistance exercise performed during long-term unloading on different indices of muscle strength, measured during actions similar or different from training tasks (paper III and V)
- the effects of long-term unloading, with or without a resistance exercise countermeasure, on surface EMG amplitude during maximal and submaximal actions (paper III and V)
- the effects of long-term unloading, with or without a resistance exercise countermeasure, on single fibre myosin heavy chain (MHC) composition of mm. vastus lateralis and soleus (paper IV)
- the effect of unloading-induced phenotype shifts on changes in muscle function, i.e. the force-velocity relationship, rate of force development and fatigability (paper V)

METHODS

GENERAL DESIGNS

Confinement study

This study, which was part of the "Simulation of Flight of International Crew on Space Station" (SFINCSS-99) study, was carried out at the Institute of Biomedical Problems (IBMP), Moscow, Russia. Four men were confined in a space station-resembling chamber at 1g for 110 days and strength training was performed 2-3 times per week. In addition, cycle ergometer exercise was carried out 2-3 times weekly on alternate days. On three or four occasions prior to confinement, subjects were familiarized with the equipment and training and testing procedures. Maximal voluntary contraction (MVC; Isometric action) was measured thrice (11, 9 and 8 days) before and twice (7 and 9 days) after confinement. Subjects gave their informed consent to the study protocol that was approved by the Human Ethics Committee at the Institute of Biomedical Problems (IBMP), Moscow, Russia.

Bed rest study

This study was part of the "Long-Term Bed Rest" study (Elmann-Larsen and Schmitt 2003, ESA-CNES-NASDA 2003a, Schmitt and Elmann-Larsen 2001) and carried out at Clinique Spatiale MEDES, Toulouse, France (Maillet and Pavy-Le Traon 1998). Nine men were subjected to 90 days of bed rest (BR), while another group of eight men (BRE) in addition performed resistance exercise for the knee extensor (Supine Squat) and plantar flexor (Calf Press) muscles every third day using a flywheel ergometer. Tests were performed over a two week period before and after the intervention. Isometric and coupled concentric-eccentric strength tests were performed and surface electromyographic (EMG) activity measured during the Supine Squat and the Calf Press in the training device prior to (two familiarization and two test sessions) and on the first (R+0) and fifth (R+4) day following bed rest. Also, rate of force development (RFD) during maximal actions and EMG amplitude during submaximal actions were measured during the Supine Squat. Peak torque during maximal one-joint, isometric, concentric and eccentric knee extensor and plantar flexor actions were measured twice before and on the second (R+1) and 11th (R+10) day after bed rest, using isokinetic dynamometry. Muscle volume was measured by means of magnetic resonance imaging (MRI) before and on day 29 and 89 during bed rest. Muscle biopsies were obtained from VL and soleus prior to and on day 84 during bed rest. Subjects gave their informed consent to the study protocol that was approved by the local Ethical Committee in Toulouse (le Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale (C.C.P.P.R.B.) de Toulouse I).

SUBJECTS

Twenty-one healthy men, 26 to 45 years and with a physical status ranging from sedentary to physically very active, participated in the two studies (Table 1).

Table 1. Anthropometrical data of subject groups in the different studies

Study	Group	n	Age (yrs)	Height (cm)	Weight (kg)
Confinement		4	38±8	176±3	77±7
Bed rest	BR	9	32±4	173±3	72±5
	BRE	8	33±5	176±5	71±6

BR = Bed rest only, BRE = Bed rest and resistance exercise.

SPACE SIMULATION MODELS

Confinement

The subjects were confined in a 200 m³ chamber (Fig. 1) for 110 days. This intervention is known to compromise normal daily locomotor activity considerably (Vaernes *et al.* 1993). Activities were similar to tasks performed on a space station and subjects had occasional interactions with “visiting crews”. They had daily contact with “ground control” outside the chamber and were monitored with the aid of a video camera. Dietary intake was set to 3480 kcal/day (Agureev and Kalandarov 2001).



Figure 1. Exterior and interior view of the confinement chamber.

Bed rest

Bed rest was performed in the 6° head-down position (Fig 2) in an effort to simulate fluid distribution and cardiovascular effects, known to occur during microgravity. Transportation, hygiene procedures and training were restricted to the head-down recumbent position. Subjects were allowed to rest on their elbows during meals and were free to move horizontally within the constraint. To ensure compliance, the volunteers were monitored at all times with video-camera surveillance and pressure-sensitive mattresses. Physiotherapy and massage were provided and ankle circumduction movements were performed daily. Subjects were provided controlled and standard diets and the caloric intake was calculated based on the weight of the subject. The intake was set to 35 kcal·kg⁻¹·day⁻¹ during the ambulatory periods prior to and following bed rest and reduced by 200 cal during bed rest. Proteins represented

12-15%, lipids 30-35% and carbohydrates 50-55% of total caloric intake (ESA-CNES-NASDA 2003a).



Figure 2. Pictures showing subjects in the 6° head-down position, maintained throughout the bed rest. Subjects were not allowed to use their lower limbs but could use their arms, e.g. while enjoying computer work or reading.

FLYWHEEL DEVICES AND TRAINING PROTOCOLS

This exercise system uses the inertia of rotating flywheel(s) to provide resistance during coupled concentric and eccentric actions (Berg and Tesch 1994, 1998). Briefly, by pulling out a strap that is wound around the axis of the flywheels (concentric action), energy is imparted to the flywheels (Fig. 3). The strap then rewinds by virtue of the energy of the rotating flywheels while the subject actively resists (eccentric action). Two flywheels of 2.5 kg each and a total inertia of 0.1105 kg·m² were used. This system works independently of gravity. In the present studies, rotational encoders i.e., ceramic hybrid sensor ball bearings (SKF AB, Gothenburg, Sweden, Model 6204-2RZTN9/HC5C3 [Study I] and Model 6206 TN9/HC5C3 [Study II-V]) situated on the axle holding the wheels, measured rotational speed. Hence, work (W ; [Flywheel Inertia · Flywheel rotational speed²] · 2⁻¹), and power (dW/dt) produced could be calculated. The ergometers were interfaced with a Windows™ based data acquisition and feedback system (MuscleLab™, Ergotest AS, Langesund, Norway) that sampled input signals at 100 Hz.

The Flywheel Multigym configuration (Fig. 4 and 6) employed in Study I, is designed for space use and allows for multiple exercises involving the upper and lower body. In the Squat, Calf Press and Back Extension a harness is used to aid in performing these tasks and in the Seated Row, Lateral Shoulder Raise, and Biceps Curl, different handles are used. The Squat and Calf Press are performed with the seat sliding. Other exercises use a fixed seat (detailed figures in paper I). When assessing MVC, force was measured with a strain-gauge (KTOYO™ 333A, KTOYO CO., LTD, Seoul, Korea) fixed between the harness and the pulley and connected to the MuscleLab™ system. Joint angles were measured by means of sensorized (knee) or manual (hip and ankle) goniometers.

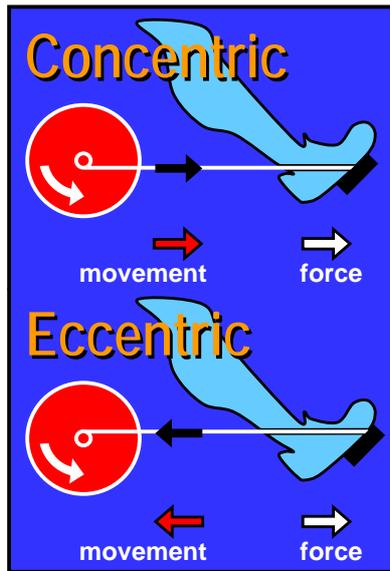


Figure 3. The principle of the flywheel device.



Figure 4. Flywheel Multigym.

For the bed rest study (paper II-V), the Flywheel Multigym was reconfigured to allow individuals performing the Supine Squat (Fig. 5 and 7) and the Calf Press in the 6° head-down position. In brief, the ergometer is suspended with rods in a sturdy frame construction, thus allowing linear movement caused by the kinetic energy transformed to the device during exercise. The entire apparatus, which rests on four wheels, is tethered with bungee cords restricting its movement. The subject is positioned on a padded back-rest, which wheels on the fixed girder and the shoulders are supported by adjustable pads with handles. Feet are restrained on the footplate with use of adjustable Velcro® straps. In the Calf Press, producing plantar flexion, the footplate provides support for the ball of the feet only. In addition to the rotational encoder, a load cell (ELA-B2E-2KL, Entran, Fairfield, NJ, USA), is mounted at the base of the pivoting foot-plate for force measurements. Moreover, knee and ankle joint angles are measured using electrogoniometers, affixed onto the right limb with a custom-built adjustable Velcro® strap system.

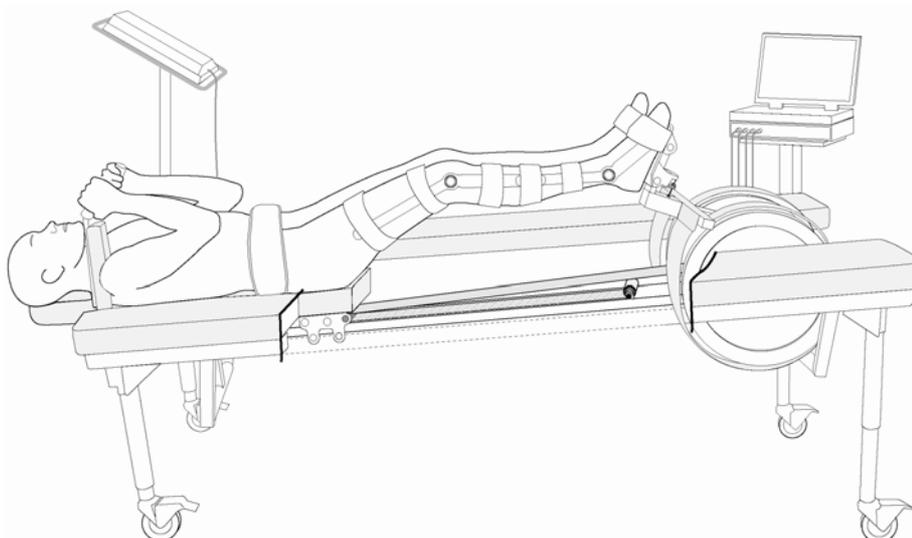


Figure 5. Drawing of the flywheel bed rest device. See text for further information.

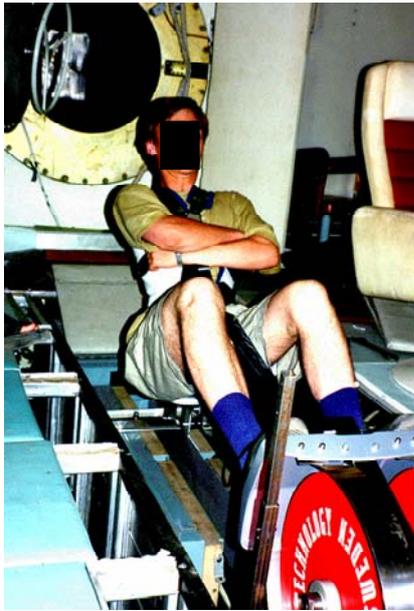


Figure 6. Flywheel training in the confinement chamber

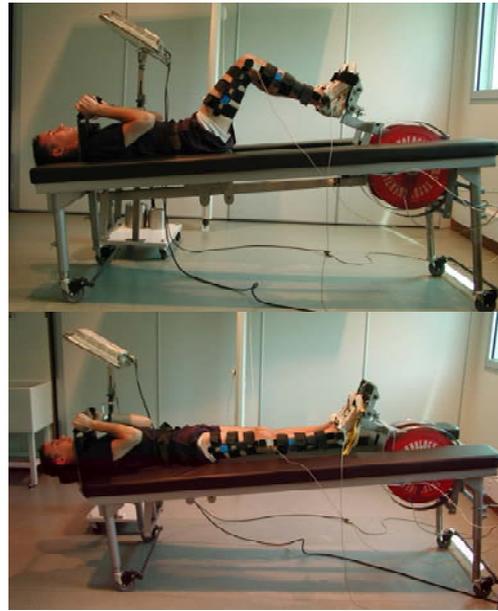


Figure 7. Subject performing the Supine Squat (upper) and the Calf Press (lower) in the bed rest flywheel device

Typical recordings of one repetition of the Supine Squat and the Calf Press are shown in Fig. 8. The test to test CV for peak force and power ranged 5.1-6.2 and 6.8-8.4% in the Supine Squat and the Calf Press, respectively. Day-to-day variation in measured maximal force is similar or even less compared to what has been reported for other training and testing devices (i.e., isokinetic dynamometry employed here, (Aagaard *et al.* 1994, Bassey and Short 1990, Holmbäck *et al.* 1999, Narici *et al.* 1989, Pavol and Grabiner 2000, Tesch *et al.* 1990, Thorstensson *et al.* 1976a, Westing *et al.* 1988) and power, work, angular velocity and minimum knee angle were also highly reproducible. Hence, this apparatus satisfies the needs of an accurate and valid device measuring voluntary force or power. EMG activity during the concentric phase in the training mode relative to MVC averaged $93 \pm 25\%$ in the Supine Squat (grand mean VL and vastus medialis; VM; $p > 0.05$) and $121 \pm 62\%$ in the Calf Press (grand mean soleus and gastrocnemius medialis; GM; $p < 0.05$). This would support that the “target” muscles are highly activated in the training task.

Resistance exercise was performed two or three times per week alternately during confinement and every third day during bed rest. In any training session, performance data (flywheel velocity in both studies and also force and angle in the bed rest study) were recorded, and work produced was displayed on a feed-back screen. All training sessions during bed rest were supervised on site. The confined subjects trained on their own, yet were guided through the exercise protocol by means of the feed-back system. They were instructed to produce at least 80% of their maximal work, traced on a computer display. Subjects in the bed rest study were encouraged to produce maximal force. Warm-up in either study consisted of progressively increased submaximal coupled concentric-eccentric actions. In the confinement study, each exercise comprised four sets of 10 repetitions. Four sets of 7 (Supine Squat) or 14 (Calf Press) repetitions were performed during bed rest. The number of repetitions in the Calf Press was twice that performed in the Supine Squat, in an effort to account for the shorter range of motion and action time.

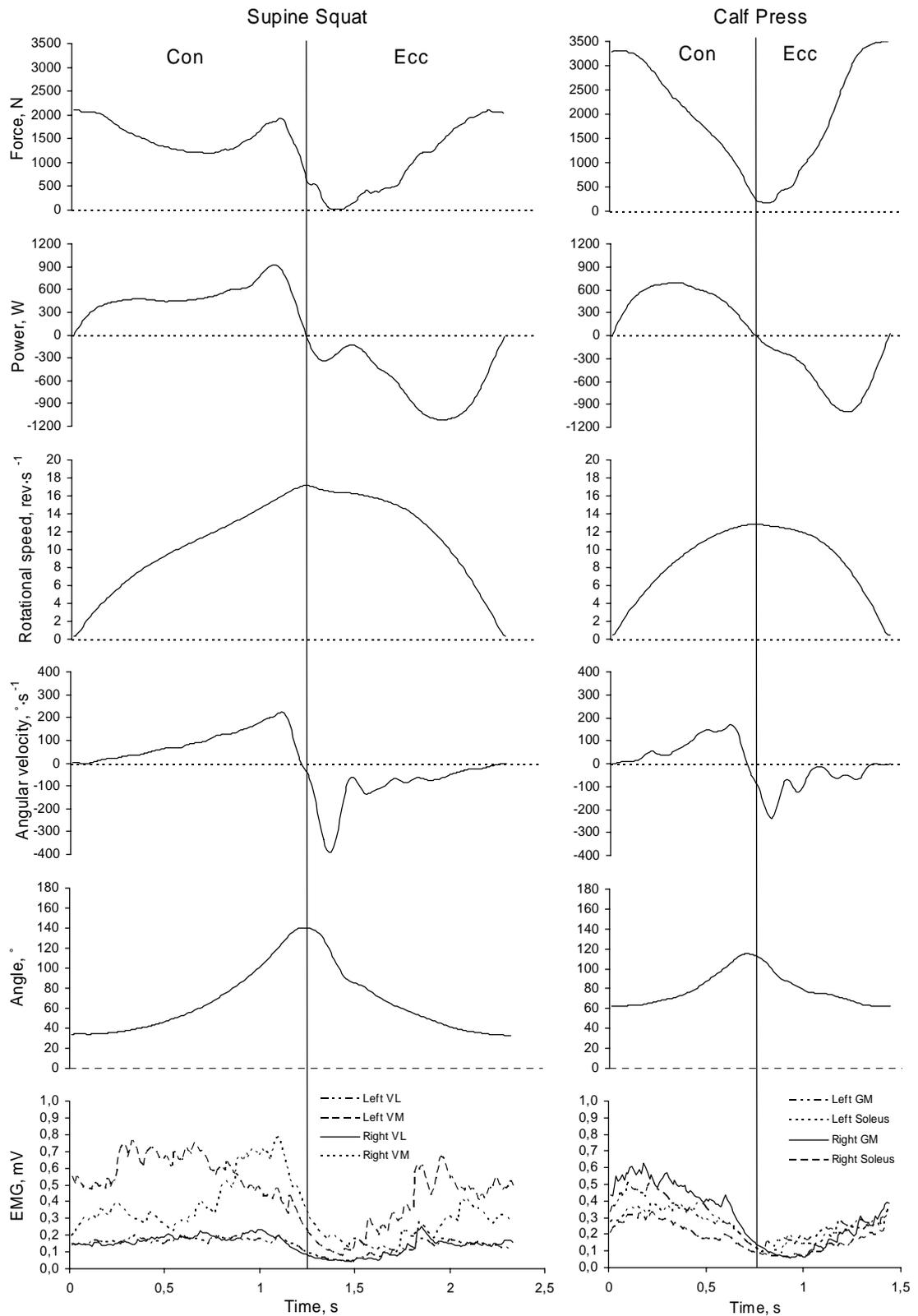


Figure 8. Typical recordings displaying force, power, rotational speed, angular velocity, joint angle and electromyographic (EMG) amplitude (RMS; Root mean square) in one repetition of a maximal coupled concentric (Con) and eccentric (Ecc) actions in the Supine Squat and Calf Press. Vertical line (—) denotes the turning point from concentric and eccentric actions. VL = Vastus Lateralis, VM = Vastus Medialis, GM = Gastrocnemius Medialis. Angle and angular velocity is for knee (Supine Squat) and ankle (Calf Press), respectively.

MUSCLE FUNCTION TESTING

Flywheel tests

In the confinement study, warm-up bouts consisting of submaximal rowing preceded MVC measurements in the Squat, Calf Press and Back Extension. In the bed rest study, all tests were performed in the 6° head-down tilt position. After warm-up bouts consisting of submaximal coupled concentric and eccentric (ConEcc) Supine Squats, MVC in Supine Squat at 90° and 120° knee joint angle were performed. These were followed by tests assessing rate of force development (RFD) and EMG activity during a sustained (60 s) submaximal (30% of MVC) isometric action and force and power in two sets of ConEcc Supine Squat. Calf Press warm-up bouts were followed by MVC in the Calf Press, performed at 90° ankle joint angle, and two sets of ConEcc Calf Press.

During MVC measurements, two trials were allowed. If force differed more than 5%, one or two additional trials were performed until no further increase. The highest value was chosen for further analyses. Subjects were instructed to increase force smoothly and maintain maximal force until halted by the investigator. Verbal encouragement, but no visual feed-back was provided during assessment of MVC. Force was averaged over the 1000 ms window showing the highest mean value.

To determine RFD, subjects performed an isometric action at 20% of pre MVC as displayed on the feedback screen and, on command from the investigator, pushed with maximal effort aiming at increase force as rapid as possible. The slope (force/time) was calculated to different time-points from the start of the action. CV was 6.4-11.8% at time points 0.2-0.5 s. During the isometric submaximal test, subjects pushed on the platform, at 30% of pre MVC for 60 s. Subjects were instructed to keep the force trace on a target line, displayed on the feedback-screen. The same absolute level was kept for the post bed rest tests. EMG amplitude (see below) was established from the 0-10 s and 50-60 s intervals together with force and joint angle signals. The EMG/force ratio was established and the difference in amplitude between the first and last 10 s period was calculated as an index of fatigue. CV for submaximal EMG measurements was 10.3-11.1%.

The dynamic tests were performed as during training. Peak force, power and joint angular velocity as well as work and minimum joint angle were determined for each repetition of sets of ConEcc Supine Squat and Calf Press actions. These values were then averaged over repetitions and the set showing the highest average force was chosen for day to day comparisons.

Isokinetic tests

Unilateral (right limb) knee extensor and plantar flexor torque was assessed using an isokinetic dynamometer (Cybex 6000[®], CYBEX International Inc., Medway, MA, USA) following a standardized submaximal warm-up on a cycle ergometer. MVC during knee extensions were performed at 90° and 120° knee joint angle. These actions were followed by concentric knee extensions at 30°, 60°, 90°, 120°, 180° and 300°·s⁻¹ and eccentric knee extensions at 30°, 90° and 120°·s⁻¹. Plantar flexions were performed in the supine position with hips and knees flexed at 90°. MVC during plantar flexion

were accomplished at 90° ankle joint angle. Concentric and eccentric plantar flexions were subsequently performed at 30 and 60 °·s⁻¹. In any mode, two trials were allowed and if peak force differed more than 5%, one or two additional trials were performed until no further increase. The highest value was chosen for further analyses. Verbal encouragement, but no visual feed-back was provided. Peak and angle-specific torque was established for each action. CV ranged 6.6-12.1 for knee extensions and 9.8-15.8 for plantar flexions.

ELECTROMYOGRAPHY

EMG activity was recorded from both limbs during the Supine Squat (VL and VM) and Calf Press (soleus and GM). After shaving and cleansing with alcohol, disposable bipolar Ag-Ag/Cl surface electrodes (Multi Bio Sensors Inc., El Paso, TX, USA) with 25 mm inter-electrode distance, were aligned longitudinally in the fibre direction. A reference electrode was placed over the tibial bone. Raw EMG signals were amplified 600 times and filtered through a band-pass filter with low and high cut-off frequencies of 6 and 1500 Hz, respectively. The filtered signal was converted to an RMS (root mean square) signal using an AD536 circuit (Analog Devices Inc., Norwood, MA, USA) with an averaging time constant of 100 ms. The converted signal, was then sampled at 100 Hz together with other input signals using the MuscleLab system (Alkner *et al.* 2000, Bosco *et al.* 1999). EMG activity during MVC was established from the 1000 ms window showing the highest mean force. EMG activity during concentric Supine Squat and Calf Press was measured over the entire action.

MUSCLE VOLUME MEASUREMENTS

The volume of m. quadriceps femoris (knee extensors) and triceps surae (plantar flexors) was measured before and on day 29 during bed rest, using magnetic resonance imaging (MRI; Siemens Somatom Impact 1.0 T, Erlangen, Germany). One hour at horizontal bed rest preceded the measurements to avoid the influence of any potential fluid shift (Berg *et al.* 1993b, Conley *et al.* 1996). Subjects also refrained from excessive muscular exercise for 24 hours before MRI. A graded foot-brace prevented compression of muscles and ensured position was reproduced across sessions. Serial scans employing a proton density sequence (TE 20.0 ms, TR 2000 ms) were obtained bilaterally from the femoral head to the knee joint and from the knee joint to the foot, respectively, as estimated from frontal scout images. Image thickness was 8 mm and the field of view was set to 480 (thigh) and 350 (calf) mm, respectively. Using computerized planimetry (Intuos Graphic Tablet, Wacom Technology, Vancouver, WA, USA), and a windows-based software program (Scion Image Beta 4.0.2 for Windows, Scion Corporation, Frederick, MD, USA), areas of interest were identified from the displayed image, manually circumscribed and then automatically computed. The three vasti muscles (VL, VM and m. vastus intermedius) were circumscribed together and m. rectus femoris (RF) separately. Every third image was measured, starting at the first image where gluteus maximus is not seen and ending with the last image where RF is seen. Triceps surae muscle volume was assessed from below caput fibulae to the last image where both heads of m. gastrocnemius were distinguished. A straight line was drawn between the anterior borders of GM and m. gastrocnemius lateralis (GL), and soleus, GM and GL were

then subsequently circumscribed. Muscle volume was calculated by summing the cross-sectional area measured in all slices (Tesch *et al.* 2004a). In addition, the sites for EMG electrode placement were identified by guidance of anatomical landmarks. The thickness of the underlying subcutaneous fat layer was subsequently measured.

Single cross-sections of the hamstrings (mm. biceps femoris, semitendinosus and semimembranosus), the adductor muscle group and the individual triceps surae muscles (soleus, GM, GL) were obtained from the left limb mid-thigh and mid-calf, respectively (unpublished data).

SINGLE FIBRE MYOSIN HEAVY CHAIN ANALYSIS

Muscle samples were obtained from VL (n=17) and soleus (n=6) using the percutaneous needle biopsy technique (Bergström 1962) about one week prior to and on day 84 during bed rest. The samples were divided into longitudinal sections and one section (~ 20 mg) from each sample was placed in a skinning solution [(in mM): 125 K propionate, 2.0 EGTA, 4.0 ATP, 1.0 MgCl₂, 20.0 imidazole (pH 7.0), and 50% (v/v) glycerol] (Trappe *et al.* 2001, Trappe *et al.* 2000) and stored at -4° C for later dissection of single muscle fibres.

The MHC isoform profile for each fibre was determined by dissecting individual fibres under a microscope and subjecting fibres to SDS-PAGE (Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis; Williamson *et al.* 2000). An average of 110 ± 5 fibres (3-4 mm in length) were studied from each of the pre and post bed rest muscle samples. The fibres were dissected in relaxing solution [(in mM) 7.0 EGTA, 20.0 imidazole, 14.5 creatine phosphate, 1.0 free Mg²⁺, 4.0 free MgATP, KCl and KOH to produce an ionic strength of 180 mM and a pH of 7.0. (pCa²⁺ 9.0)] and then solubilized in 80 µl of 10% SDS sample buffer [10% SDS, 6 mg/ml EDTA, 0.06 M Tris (pH 6.8), 2 mg/ml bromphenol blue, 15% glycerol, and 5% b-mercaptoethanol]. These samples were stored at -80°C until analyzed for MHC content using SDS-PAGE (SE 600 series, Hoefer, San Francisco, CA).

Samples were loaded on a 3.5% loading and a 5% separating gel and ran for about 14 hours at 4°C. The gels were silver stained, revealing the MHC isoform profile for each individual fibre. MHC protein expression distribution was identified according to migration rates and compared with molecular weight standards of each single fibre. For any individual fibre, MHC was categorized as MHC I, IIa, IIx, I/IIa, I/IIa/IIx or IIa/IIx. Individual MHC fibre-type distribution was determined from the about 110 fibres per biopsy pre and post bed rest, respectively. From these fibres, the percentage of the total for each MHC isoform was represented. The inter- and intra-assay variability of this technique has been shown to be less than 0.1% (Williamson *et al.* 2000). Furthermore, the coefficient of determination (r²), comparing the MHC distribution of 100 fibres vs. another 100 fibres from the same biopsy sample, was 0.960 (Williamson *et al.* 2000).

STATISTICS

Data are mean \pm SD. Due to the low number of subjects in the confinement study (n=4), individual data are presented and no statistics were performed. In the bed rest study, day to day variation (coefficient of variation; CV %) was calculated by dividing the SD by the mean and multiplying by 100 for each individual $[(SD \cdot mean^{-1}) \cdot 100]$, and averaging across subjects (n=23; additional subjects were tested when assessing CV, see paper II). A repeated measures, two-factorial ANOVA (Analysis of Variance) was used to detect group*time interaction to compare the groups for each parameter. To identify differences in responses between muscles, the relative changes from baseline to d 29 and 89 were used for comparisons and a repeated measures, three-factorial ANOVA was employed to detect muscle*group*time interaction. Similarly, differences between the relative changes in torque at different isokinetic velocities were calculated. When significant interactions were seen, planned comparisons were performed to detect where differences occurred. Changes over time were analysed for each group. Bonferroni corrections were made for each level of comparisons. Changes in performance during training between the first and last five sessions, and differences in EMG activity between actions, were detected using Students t-test. Statistical significance was set to $p < 0.05$ for all analyses.

RESULTS

TRAINING

The training load for the six exercises and four subjects either increased or was maintained during confinement (Fig. 9). The relative increase in work (i.e., the grand mean of the last five compared to the initial five training sessions) for the six exercises averaged (range): Squat: 62% (24-109%); Calf Press: 58% (3-116%); Back Extension: 53% ([-3]-97%); Seated Row: 61% (11-119%); Shoulder Raise: 58% (18-112%) and Biceps Curl: 143% (55-238%). One subject decided to refrain from exercises involving the back (i.e., Squat, Calf Press and Back Extension) during day 54-76 of the confinement due to back pain. Other subjects adhered to the protocol and withdraw only on a few occasions due to minor pain or soreness. Using a five-graded scale (from very poor to very good) comfort was assessed and was rated to be adequate, good or very good (3-5) for all exercises except Back Extension, that due to pain problems was rated very poor or poor (1-2) by two subjects. However, comfort increased over the course of the intervention.

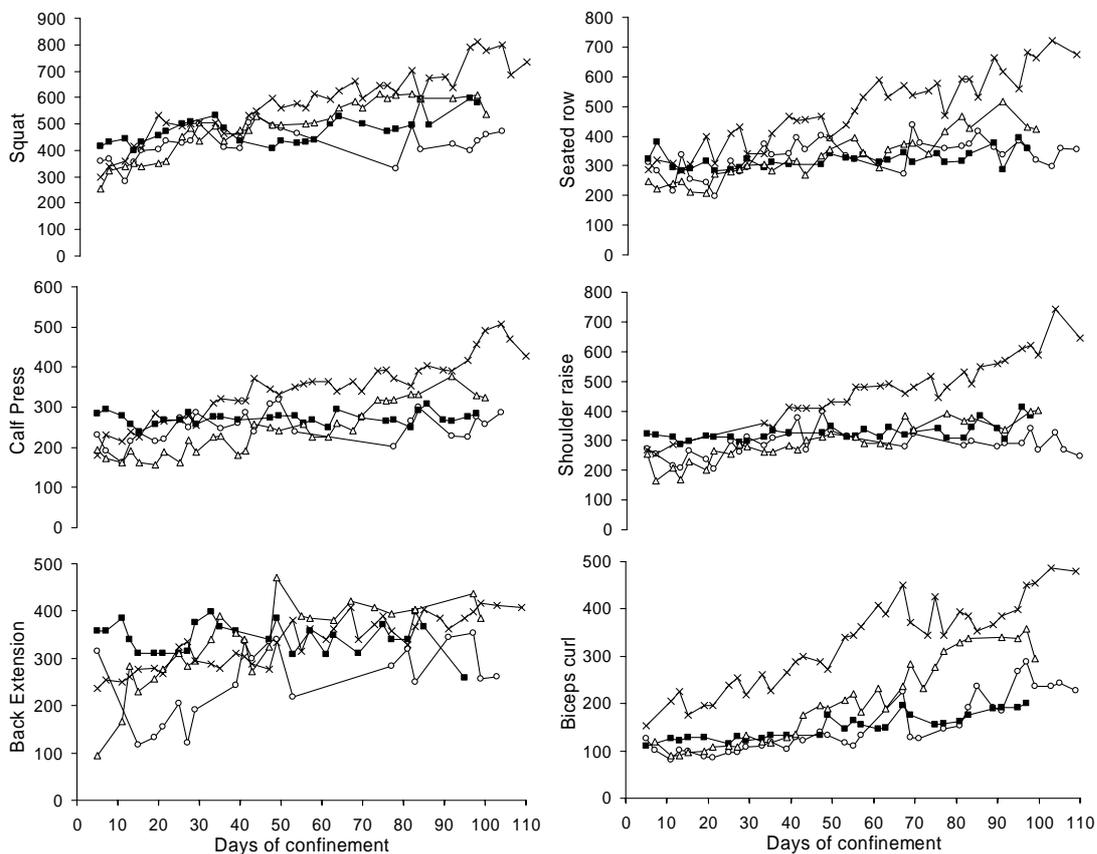


Figure 9. Work (J) averaged over repetitions and sets for each exercise session and subject during the course of confinement.

Training performance indices (concentric and eccentric force, power, work and angular velocity during training), were maintained ($p>0.05$) over the course of the bed rest for both the Supine Squat and Calf Press (Fig. 10). Minimum knee angle (turning point between eccentric and concentric action) and range of motion were unaltered ($p>0.05$) suggesting maintained strategy when performing the task. The subjects complied with the training protocol; only a total of five out of 464 sessions (Supine Squat or Calf Press) were cancelled due to temporary pain or soreness.

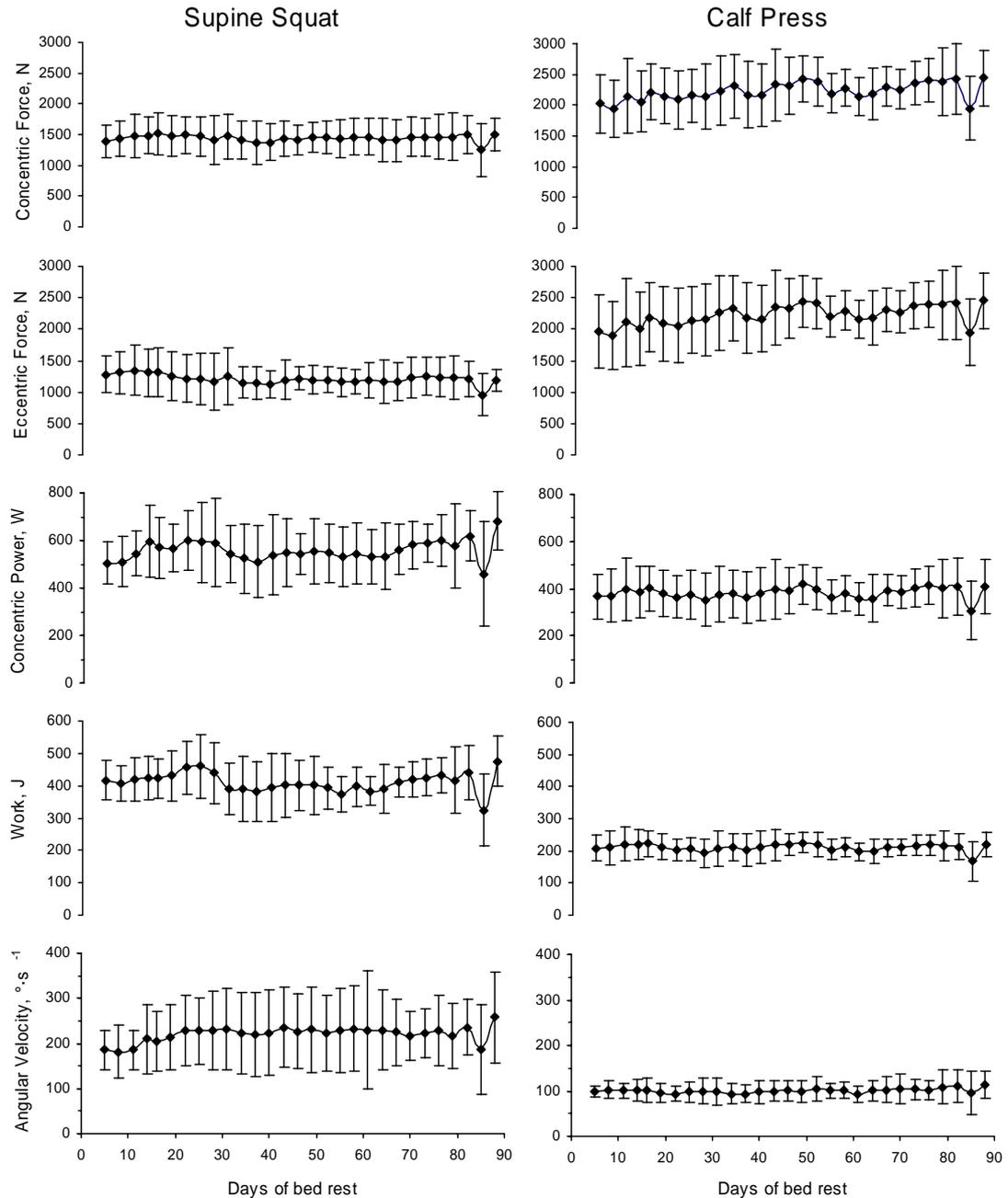


Figure 10. Concentric and eccentric force, concentric power, work and angular velocity obtained during training sessions in the Supine Squat and Calf Press performed during bed rest. Each data point is mean and SD and represents peak values averaged over repetitions and sets.

MUSCLE FUNCTION

Isometric strength in the Squat and the Calf Press either increased or was maintained during the 110 day confinement. Whereas two subjects maintained strength in the Back Extension, two subjects showed a decrease (Fig. 11).

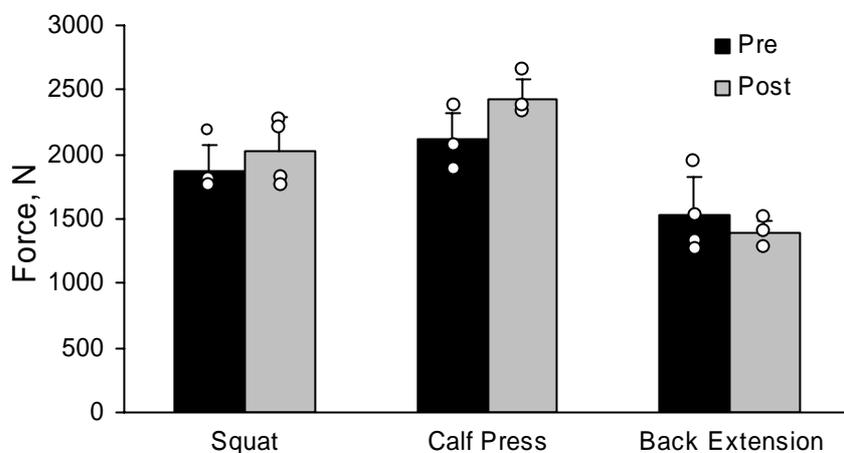


Figure 11. Isometric force Pre and Post confinement. Values are mean +SD and are averaged over angles. White dots represent individual data points of the four subjects, sometimes overlapping each other.

Relative changes in various measures of maximal strength in response to bed rest are illustrated in Fig. 12. In brief, any strength parameter measured using flywheel or isokinetic dynamometry decreased ($p < 0.05$) in BR for both muscle groups. BRE maintained ($p > 0.05$) training-specific performance in the flywheel for the two exercises, and even increased concentric and eccentric force in the Calf Press R+4 ($p < 0.05$). MVC was maintained ($p > 0.05$) in the Supine Squat but not ($p < 0.05$) in the Calf Press. Peak torque during isokinetic dynamometry decreased ($p < 0.05$) regardless of mode. However, the decrease in knee extension peak torque was less ($p < 0.05$) in BRE than in BR at R+1.

There was no difference between groups regarding minimum knee angle and range of motion. Neither group showed a change ($p > 0.05$) in any parameter measured in the flywheel from R+0 to R+4. BR showed increased ($p < 0.05$) isokinetic peak torque from R+1 to R+10. BRE increased ($p < 0.05$) MVC at 90° in knee extension and eccentric plantar flexion tasks. There was no difference ($p > 0.05$) in response between muscles regarding muscle function except for MVC measured in the isokinetic device; knee extension showed greater ($p < 0.05$) decrease than plantar flexion in BR.

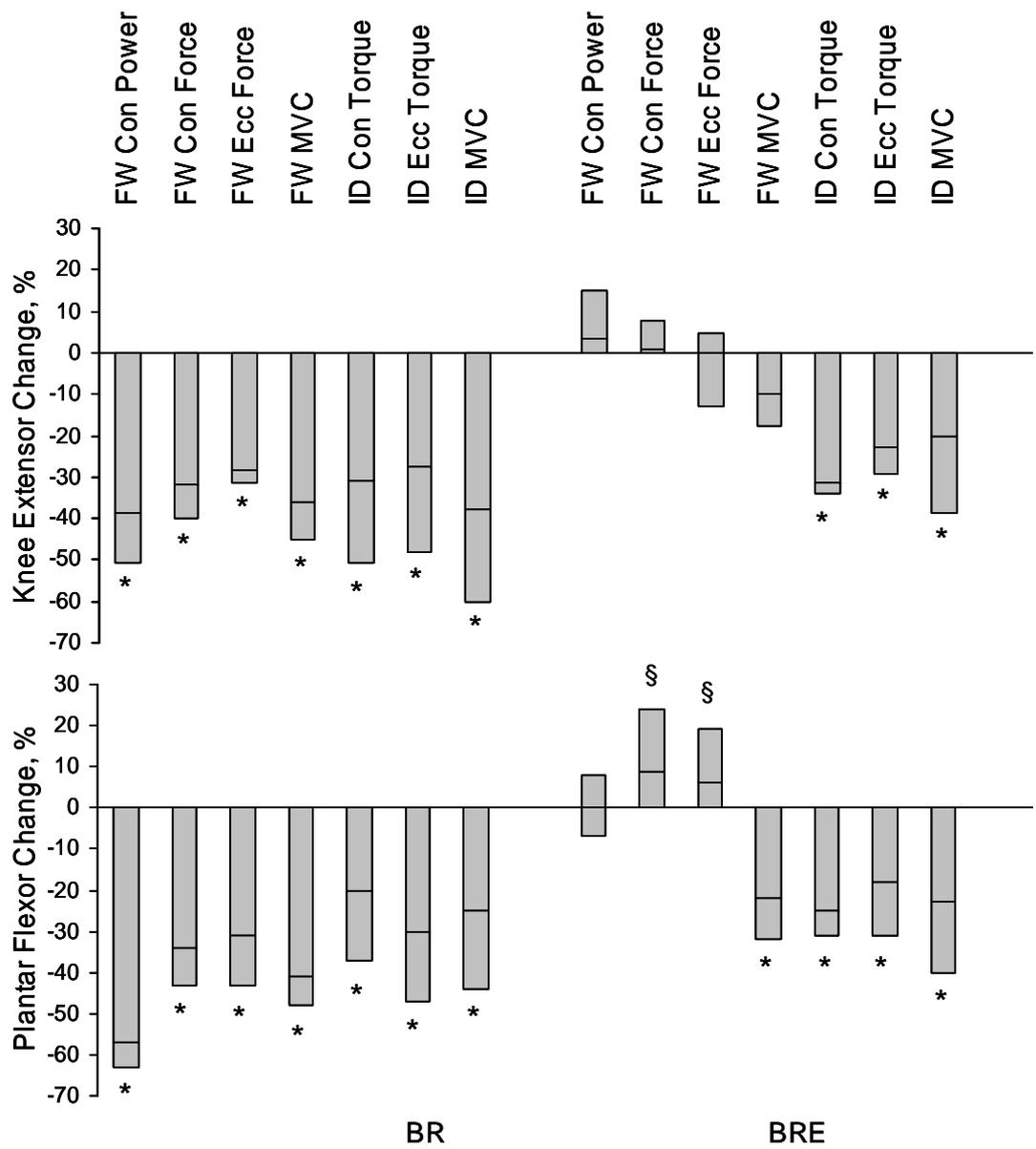


Figure 12. Relative changes in indices of maximal strength for the knee extensors (upper graph) and plantar flexors (lower graph) using the flywheel device (FW) or isokinetic dynamometry (ID). Flywheel tests are performed before and on the first (R+0) and fifth (R+4) day after bed rest. Isokinetic tests are performed before and on the second (R+1) and 11th (R+10) day following bed rest. Lower line represents first post measurement and upper line the second. BR = bed rest only, BRE = bed rest with exercise countermeasure. Con = Concentric, Ecc = Eccentric, MVC = Maximal Voluntary Contraction (Isometric). * denotes difference ($p < 0.05$) at both post measurements and § only at the second post test.

The torque-velocity curves for knee extension are shown in Fig. 13. There was a decrease in angle-specific torque (120° knee angle) for all velocities and groups from pre to R+1 and R+10. No significant interaction was detected between velocities.

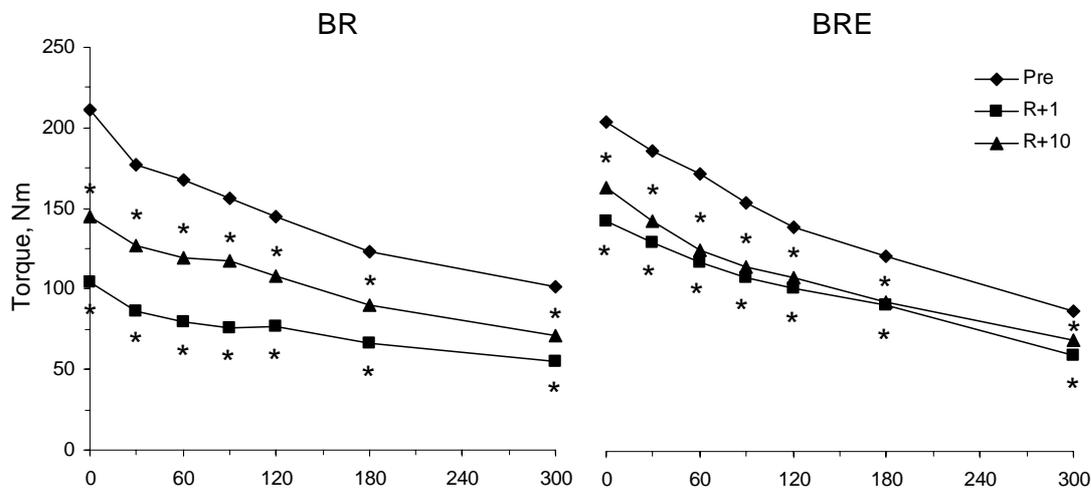


Figure 13. Torque-velocity relationship measured by means of isokinetic dynamometry. Values are torque at 120° knee angle. Data points are mean. BR = Bed rest only, BRE = Bed rest and resistance exercise countermeasure, R+1 = Second day of ambulation, R+10 = Eleventh day of ambulation. * denotes significant difference ($p < 0.05$) from pre.

Rate of force development (RFD) decreased at all time points (0.2-0.5 s) on day R+0 and R+4 in BR (Fig. 14). BRE showed a decrease on R+0 but had returned to baseline on R+4. The decrease was greater ($p < 0.05$) for group BR at all time points. When calculating the slope normalized to maximal force, neither group showed any change, except that a reduction was seen in BR at 0.2 s (R+0 and R+4) and 0.5 s (R+4), and in BRE at 0.2 s (R+0).

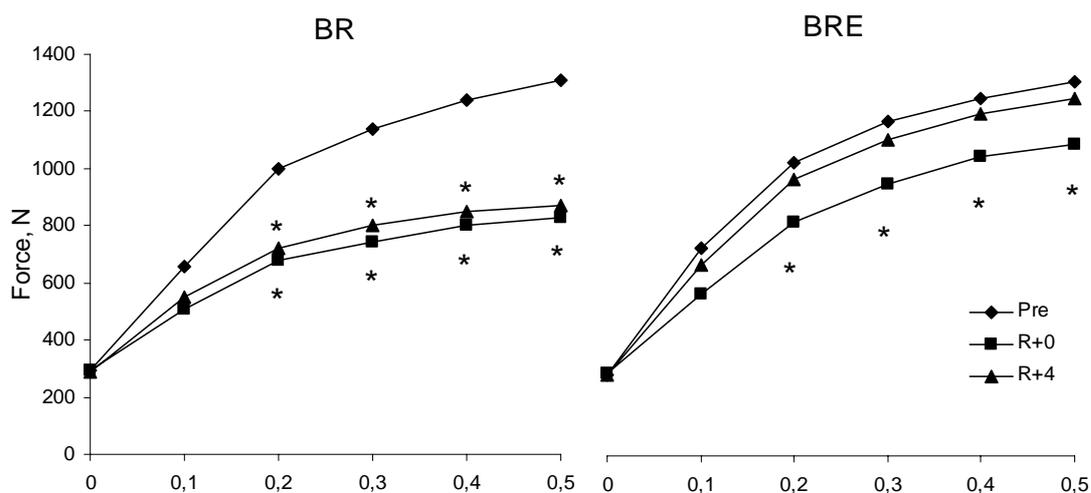


Figure 14. Rate of force development (RFD) measured from a starting level of 20% of pre MVC at five different time points during an isometric action. Data points are mean. BR = Bed rest only, BRE = Bed rest and resistance exercise countermeasure, R+0 = First day of ambulation, R+4 = Fifth day of ambulation. * denotes significant difference ($p < 0.05$) from pre.

ELECTROMYOGRAPHY

EMG data obtained from VL and VM during maximal or submaximal isometric Supine Squat at 90° knee angle are summarized in Fig. 15. Maximal quadriceps EMG amplitude decreased ($p < 0.05$) to R+0 in BR but was maintained ($p > 0.05$) in BRE (also at 120° knee angle, data not shown). In the submaximal test, EMG amplitude was greater in BR ($p < 0.05$) for both time windows (0-10 s and 50-60 s) following bed rest, while BRE maintained ($p > 0.05$) the same amplitude. The increase in EMG amplitude over the 60 s isometric action was potentiated ($p < 0.05$) on R+0 and R+4 compared to Pre in BR. BRE showed no alteration in this response ($p > 0.05$). Concentric EMG in the Supine Squat decreased ($p < 0.05$) for BR but was maintained for BRE. Maximal amplitude for the plantar flexors decreased ($p < 0.05$) for all measurements except for MVC at R+0 in BR. BRE maintained EMG amplitude in all measurements.

The subcutaneous fat layer, which is known to alter the EMG signal (Farina *et al.* 2002, Nordander *et al.* 2003), was unchanged over the electrode sites for both muscle groups examined.

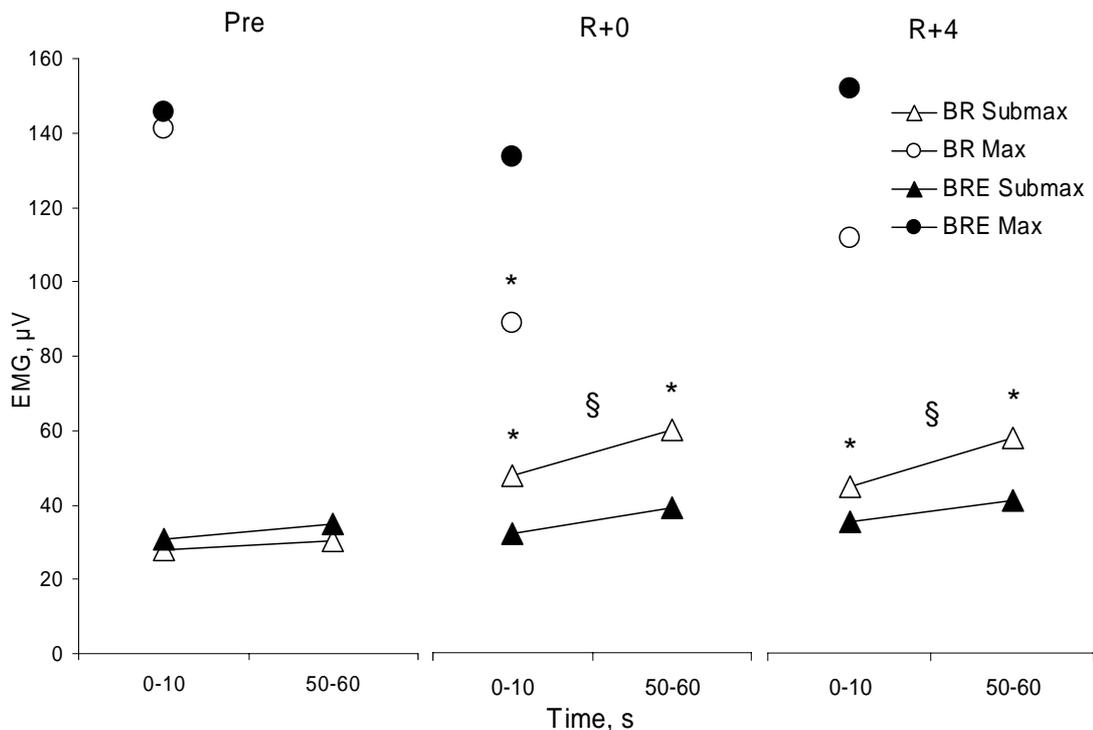


Figure 15. EMG amplitude averaged over vastus lateralis and medialis bilaterally. Data points are mean. Maximal data are averaged over 1 s during maximal voluntary contraction (MVC) in the Supine Squat position at 90° knee angle. Submaximal data are averaged over the first (0-10) and last (50-60) 10 s-periods of a 60 s sustained contraction at 30% of MVC as measured before bed rest. BR = Bed rest only, BRE = Bed rest and resistance exercise countermeasure, R+0 = First day of ambulation, R+4 = Fifth day of ambulation. * denotes a change ($p < 0.05$) from pre in absolute EMG amplitude, § denotes a change ($p < 0.05$) from pre in increase between the two time periods.

MUSCLE VOLUME

Quadriceps muscle volume decreased ($p < 0.05$) by 10% on day 29 and by 18% day 89 in group BR (Fig. 16). The vasti muscles showed a 10 % decrease ($p < 0.05$) day 29 and a 19% decrease day 89. Rectus femoris (RF) muscle volume was maintained ($p > 0.05$) on day 29 but had decreased 9% ($p < 0.05$) on day 89. BRE showed no change for any muscle ($p > 0.05$). The atrophy was greater ($p < 0.05$) in BR for all muscles except for RF at day 29. Triceps surae muscle volume decreased ($p < 0.05$) by 16% day 29 and by 29% day 89 in BR and by 8 and 15% respectively in BRE (Fig. 17). This decrease was greater in BR than in BRE ($p < 0.05$). The atrophy was greater ($p < 0.05$) for the plantar flexors than for the knee extensor in both subject groups.

In BR, the individual plantar flexor muscles showed a somewhat different response such that soleus (-16 and -31) and GM (-16 and -29%) showed greater atrophy than GL (-12 and -24). This difference was significant only on day 29. In BRE, soleus (-12 and -24) atrophied more ($p < 0.05$) than GM (-5 and -12) and GL (no significant change).

The hamstring muscle group atrophied ($p < 0.05$) by 7 and 13% in BR and by 6 and 11% in BRE, at day 29 and 89 respectively. The adductor muscle group atrophied ($p < 0.05$) 7 and 13% in BR and 6 and 7% in BRE, at day 29 and 89 respectively. Gracilis muscle volume did not change over time for any group. There was no difference in atrophy between the groups for the hamstring and adductor muscle groups ($p > 0.05$).

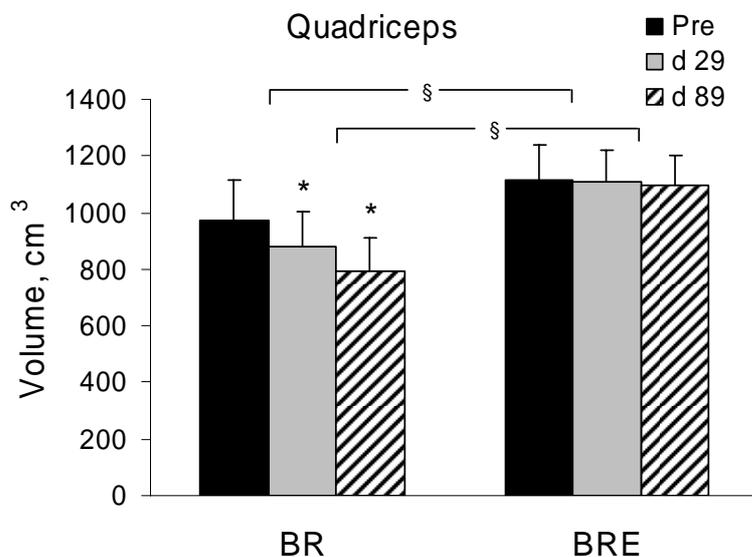


Figure 16. Quadriceps femoris muscle volume obtained by means of MRI prior to (pre) and on day 29 and 89 during bed rest. BR = Bed rest only, BRE = Bed rest and resistance exercise countermeasure. * denotes change ($p < 0.05$) from pre, § denotes difference ($p < 0.05$) between groups regarding the change.

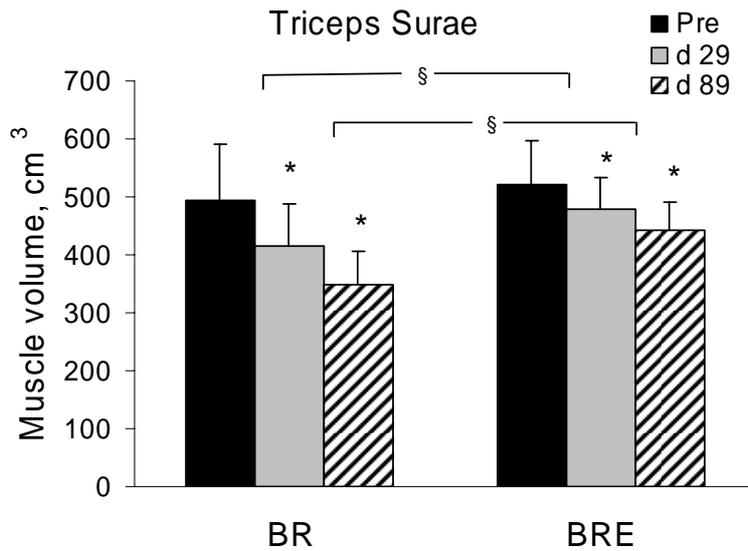


Figure 17. Triceps Surae muscle volume obtained by means of MRI prior to (pre) and on day 29 and 89 during bed rest. BR = Bed rest only, BRE = Bed rest and resistance exercise countermeasure. * denotes change ($p < 0.05$) from pre, § denotes difference ($p < 0.05$) between groups regarding the change.

MYOSIN HEAVY CHAIN DISTRIBUTION

Both BR and BRE showed a decrease ($p < 0.05$) in MHC I fibres of 15-16% while only BR had a decrease in MHC IIa fibres (-14%; Fig. 18). Both BR and BRE had more hybrid fibres following bed rest ($p < 0.05$) and the increase was greater ($p < 0.05$) in BR (+29%) than in BRE (+12%). For the individual hybrid fibre types, there were increases in MHC I/IIa, IIa/IIx and I/IIa/IIx fibres in BR, but not in BRE.

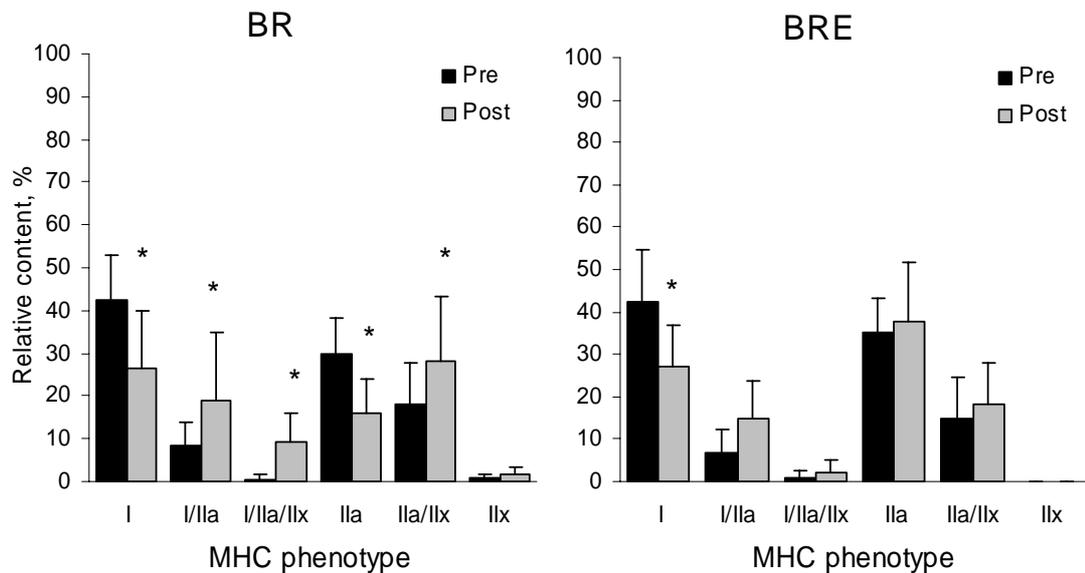


Figure 18. Myosin heavy chain (MHC) distribution in the vastus lateralis muscle Pre and 84 days Post bed rest. BR = Bed rest only ($n=9$), BRE = Bed rest and resistance exercise countermeasure ($n=8$). Data presented as mean + SD. * denotes significant difference between pre and post values ($p < 0.05$).

In soleus, there was a 19% decrease ($p < 0.05$) in MHC I fibres and a 22% increase in total hybrid fibres ($p < 0.05$) in BR (Fig. 19). However, there was no change in any of the individual hybrid fibre types that reached statistical significance. No changes were observed in MHC distribution in BRE.

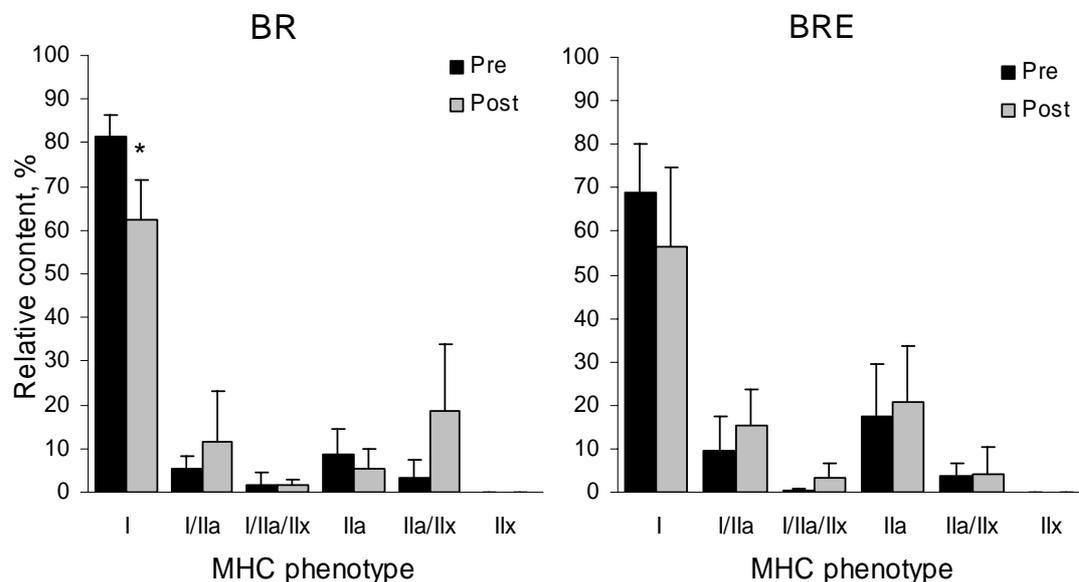


Figure 19. Myosin heavy chain (MHC) distribution in the soleus muscle Pre and 84 days Post bed rest. BR = Bed rest only (n=3), BRE = Bed rest and resistance exercise countermeasure (n=3). Data presented as mean + SD. * denotes significant difference between pre and post values ($p < 0.05$).

MONITORING AND FOLLOW-UP

All subjects complied with the bed rest protocol. No subject experienced clinically significant urinary lithiases, a possible side-effect of bed rest (Hwang *et al.* 1988). It is generally held that thromboembolism is a serious consequence of bed rest. However, no evidence of increased risk in healthy bedridden subjects has been documented (Greenleaf *et al.* 2004), and no thrombosis was detected in the volunteers of the present study. There were temporary episodes of muscular pain due to bed rest, training and re-ambulation, but none of persistent nature. Follow-up visits were performed up to 360 days upon completion of bed rest and revealed no complications or impairments of clinical significance (cf. ESA-CNES-NASDA 2003b).

DISCUSSION

The present thesis shows that the marked muscle atrophy and dysfunction in response to long-term unloading are offset or blunted with short episodes of resistance exercise. While it is evident that the plantar flexor muscles are more vulnerable to unloading than the knee extensor muscles, as reflected in more pronounced atrophy, it is also clear that both muscle groups benefit from resistance exercise promoting neuronal adaptations and modified protein metabolism.

The current investigation has established that long-term unloading leads to changes in muscle phenotype favouring a slow to fast MHC shift that is attenuated by resistance exercise. These changes are not associated with altered *in-vivo* force-velocity characteristics. It seems that neural adaptations, in addition to changes in muscle size, are responsible for muscle function deterioration. Hence, maintaining muscle function and complex motor tasks in the absence of weight bearing activities, e.g., bed rest and spaceflight, extends beyond preserving muscle size only.

EFFECTS OF BED REST ON MUSCLE

The data reported here clearly show a greater atrophy for the plantar flexors compared to the knee extensors following bed rest. The results of past human studies in this context are not conclusive (Akima *et al.* 2001, Berry *et al.* 1993, Convertino *et al.* 1989, Ferrando *et al.* 1995, Hather *et al.* 1992, LeBlanc *et al.* 1992, Schulze *et al.* 2002, Tesch *et al.* 2004b), but when combined, support the current finding (Fig. 20). Overall, plantar flexor muscles contain a larger portion of slow (type I) fibres than the knee extensor muscles (Gollnick *et al.* 1974b, Johnson *et al.* 1973). In the subjects of this study, there was a tendency towards preferential slow fibre atrophy (Chopard *et al.* 2005, Rudnick *et al.* 2004, Trappe *et al.* 2004a) following BR. A greater atrophy in type I than type II appeared evident in patients following trauma (Edström 1970, Häggmark and Eriksson 1979, Häggmark *et al.* 1981), but not in healthy individuals subjected to 16-30 days unloading (Adams *et al.* 1994, Berg *et al.* 1993a, Hikida *et al.* 1989). Surprisingly and in frank contrast to the current data, short-term (<17 days) spaceflight provoked greater type II fibre atrophy (Edgerton *et al.* 1995, Widrick *et al.* 1999). Nevertheless, the present long-term data are not surprising, given that slow fibres are more frequently used in sustained low force tasks (Gollnick *et al.* 1974a) and that there is a greater reliance upon the plantar flexor muscle group in daily locomotor activities (Ericson *et al.* 1986, Winter and Yack 1987). Also, weight-bearing is more pronounced in the plantar flexors compared to the knee extensors. Thus, the “slow” plantar flexors may simply be more prone to undergo atrophy when unloaded. GL, that is less active than GM and soleus during walking (Ericson *et al.* 1986), showed less atrophic response to bed rest. This fact makes it attractive to speculate that muscles that are more used in everyday life are more susceptible to unloading. Moreover, and in agreement with most previous studies (Adams *et al.* 1994, Deitrick *et al.* 1948, Hather *et al.* 1992, Shackelford *et al.* 2004), the non-weight-bearing hamstring and adductor muscles exhibited less atrophy than the anti-gravity muscles.

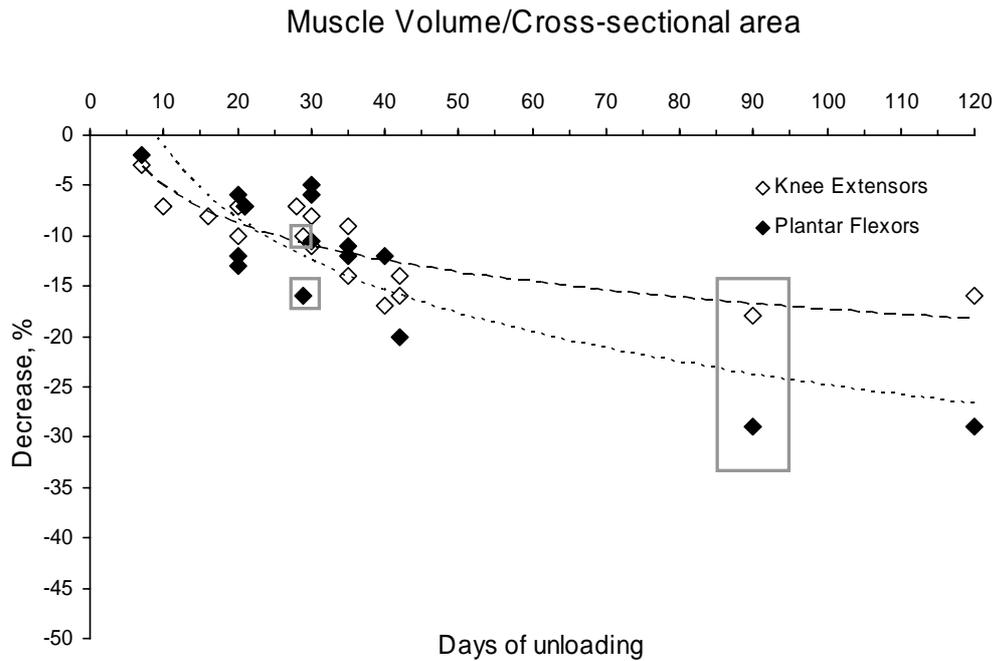


Figure 20. Relative atrophy for the knee extensor and plantar flexor muscles obtained at different time-points in the current and previous studies (Adams *et al.* 1994, Akima *et al.* 2001, Akima *et al.* 2003, Berg *et al.* 1991, Berg *et al.* 1997, Berry *et al.* 1993, Caruso *et al.* 2005, Caruso *et al.* 2004, Convertino *et al.* 1989, Ferrando *et al.* 1995, Greenleaf 1997, Hather *et al.* 1992, LeBlanc *et al.* 1988, Ploutz-Snyder *et al.* 1995, Schulze *et al.* 2002, Shackelford *et al.* 2004, Suzuki *et al.* 1994, Tesch *et al.* 2004b). Symbols in grey squares are current data. Best logarithmic curve fit is denoted by a dashed line for knee extensors and a dotted line for plantar flexors.

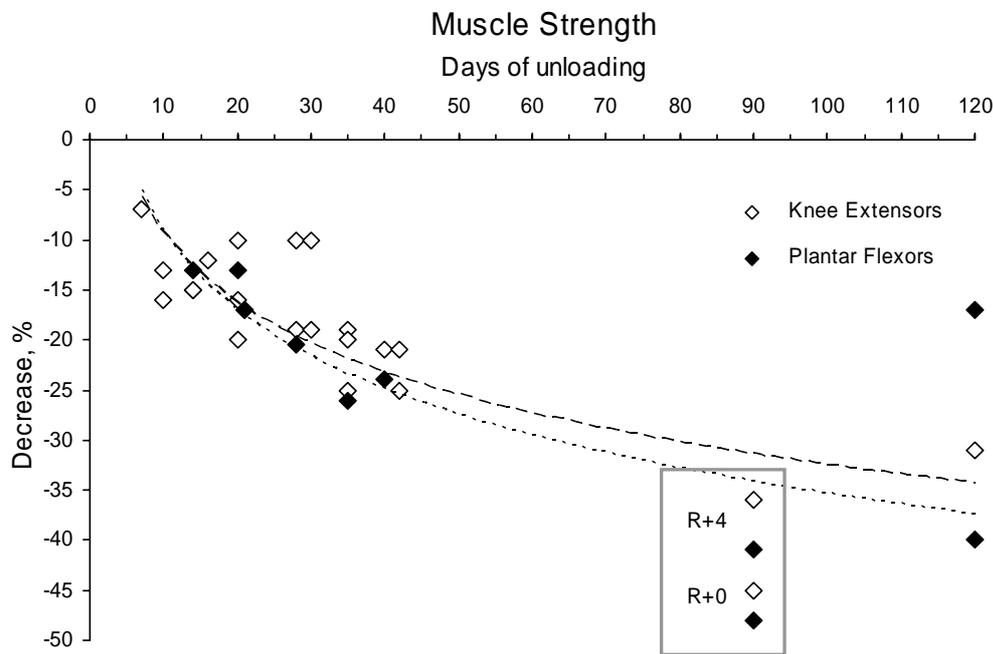


Figure 21. Relative decrease in maximal strength for the knee extensor and plantar flexor muscles obtained at different time-points in the current and previous studies (Adams *et al.* 1994, Akima *et al.* 2001, Bamman *et al.* 1998, Bamman *et al.* 1997, Berg *et al.* 1991, Berg *et al.* 1997, Berg and Tesch 1996, Caruso *et al.* 2005, Caruso *et al.* 2004, Dudley *et al.* 1992, Dudley *et al.* 1989, Funato *et al.* 1997, Germain *et al.* 1995, Gogja *et al.* 1988, Greenleaf 1997, Koryak 1999a, LeBlanc *et al.* 1992, Ploutz-Snyder *et al.* 1995, Portero *et al.* 1996, Schulze *et al.* 2002, Suzuki *et al.* 1994, Tesch *et al.* 2004b). Symbols in grey square are current data. Best logarithmic curve fit is denoted by a dashed line for knee extensors and a dotted line for plantar flexors.

The magnitude of both knee extensor and plantar flexor muscle atrophy during the first month was similar to that during the subsequent two months of bed rest, i.e., the estimated rate of atrophy was reduced with time. Data from previous short-term studies not exceeding six weeks, suggest that atrophy occurs at a rather steady rate. However, current and previous long-term data suggest that the rate of atrophy levels off with extended unloading (Fig. 20).

The findings reported here and elsewhere (Fig. 20 and 21; Adams *et al.* 1994, Akima *et al.* 2001, Berg *et al.* 1991, Berg *et al.* 1997, Convertino *et al.* 1989, Dudley *et al.* 1989, LeBlanc *et al.* 1992, Ploutz-Snyder *et al.* 1995, Schulze *et al.* 2002, Suzuki *et al.* 1994, Tesch *et al.* 2004b), which convincingly show that the decrease in maximal voluntary strength cannot be accounted for by muscle atrophy only, are evidence that additional factors contribute to the impaired muscle function demonstrated after unloading.

Similar to findings reported following short-term unloading (Berg *et al.* 1997, Dudley *et al.* 1992), EMG amplitude during maximal effort was reduced following bed rest. This would infer impaired ability to activate muscles by voluntary means, hence decrease in maximal voluntary strength. The fact that EMG amplitude decreased during maximal effort, yet showed a marked increase during a low force standardized isometric action, strongly supports that there were true alterations in muscle activation. It is attractive to attribute these results and those showing greater impairment in voluntary than electrically evoked force following bed rest (Duchateau 1995, Koryak 1995a, 1996), and reduced force with no atrophy following plaster immobilization (Deschenes *et al.* 2002), to a decreased neural drive.

In the subjects of the present bed rest study, the intrinsic properties of the VL muscle were modified. Thus, specific force and power decreased in single fibres (Trappe *et al.* 2004a). If this effect translates over to *in-vivo* whole muscle function, it could in part explain the decrease in maximal strength and the increased EMG amplitude noted during submaximal action to compensate for the loss in the single fibre contractile force.

The current study also demonstrated significant changes in MHC composition for both VL and soleus and favouring a slow to fast shift that previous short-term studies have failed to show (Adams *et al.* 1994, Bamman *et al.* 1998, Berg *et al.* 1993a, Berg *et al.* 1997, Ferretti *et al.* 1997, Hikida *et al.* 1989). Collectively, from these studies it appears that only extended periods of unloading will induce such an effect. In support, there was an increase in the number of fibres that were in transition from slow to fast fibres at the mRNA level, with no obvious changes at the protein level following 37 days of bed rest (Andersen *et al.* 1999). Similar to what has been shown following detraining (Andersen and Aagaard 2000), there was an increase in fibres containing MHC IIx, suggesting this being the “default gene”, expressed when no training is performed (Adams *et al.* 1993, Andersen and Aagaard 2000, Andersen *et al.* 1994).

A correlation between MHC composition and *in vivo* muscle function was evident when subjects with different phenotype profiles were studied (Aagaard and Andersen 1998, Gür *et al.* 2003, Harridge *et al.* 1996). A concurrent change in MHC composition and *in vivo* force-velocity relationship was also reported following detraining after chronic resistance exercise (Andersen *et al.* 2005). Neither the current nor previous short-term unloading studies, showing no effect on fibre-type composition (Berg *et al.*

1997, Dudley *et al.* 1989, Hikida *et al.* 1989), demonstrated altered force-velocity relationship. Moreover and concerting previous studies (Koryak 1995a, 1998a, 1999b), RFD normalized to maximal force, did not show the increase that could be expected as a result of a slow-to-fast phenotype shift. It therefore appears that the effect of this shift is overridden by the effects of the general decrease in specific power of single fibres (Trappe *et al.* 2004a) and/or impaired motor unit recruitment and activation. Altogether, the current data suggest that even significant changes in muscle phenotype following unloading, elicit subtle, if any, changes in *in-vivo* muscle function.

EMG amplitude of the knee extensor muscles increased during a sustained submaximal isometric action, inferring increased fatigability (Cobb and Forbes 1923, Petrofsky and Lind 1980). Exacerbated fatigue when subjected to a sustained isometric action at a fixed load has also been reported for the plantar flexors following four weeks of bed rest (Portero *et al.* 1996). The increased fatigability may in part be explained by the increase in relative workload (Monod 1956, Rohmert 1960), as a consequence of the decreased MVC following bed rest. Hence, producing a given force, calls for additional, preferentially high-threshold, fast motor units to be recruited (Gollnick *et al.* 1974a) following bed rest. This, in turn would give rise to increased surface EMG amplitude (Berg and Tesch 1996, Kozlovskaya *et al.* 1981). The altered phenotype composition towards more fatigable fibres may have augmented the fatigue response as well. Given that this effect was not seen in BRE, which also exhibited a slow-to-fast phenotype shift, would suggest that modifications in motor control, directly or indirectly as a consequence of atrophy, were responsible for the exaggerated fatigue response after bed rest.

RESISTANCE EXERCISE COUNTERMEASURE

This work examined a potential countermeasure to muscle atrophy and function loss during spaceflight. A gravity-independent flywheel ergometer (Berg and Tesch 1994, Tesch *et al.* 2004a, Tesch *et al.* 2004b) was proven effective as a resistance exercise device in ambulatory and unloaded subjects (Berg and Tesch 1994, Tesch *et al.* 2004a, Tesch *et al.* 2004b). Prior to implementing this particular exercise paradigm in a long-term bed rest study, its feasibility and efficacy were validated under space station-like conditions (paper I). Individuals confined for four months in a ground-based space station replica, performed resistance exercise using a configuration designed for space. Overall training performance showed marked progress and MVC increased or was maintained. Furthermore, VL muscle fibre area increased in these individuals (Kozlovskaya 2000), suggesting that the training program produced muscle hypertrophy. Though confinement does not provoke chronic muscle unloading, locomotor activity is severely restricted (Vaernes *et al.* 1993). Despite the few subjects examined and the lack of control subjects undergoing confinement only, this study provides unique data on muscle function adaptations under controlled long-term space station-like conditions.

The results from the 90 day bed rest study clearly show that resistance exercise can prevent muscle atrophy and muscle function impairments induced by long-term unloading. The 18% knee extensor muscle atrophy following bed rest was offset by resistance exercise performed every third day. Previously, knee extensor muscle function and size was maintained during unloading studies not exceeding 5 weeks, and

employing similar or more frequent resistance exercise protocols (Akima *et al.* 2001, Bamman *et al.* 1998, Schulze *et al.* 2002, Tesch *et al.* 2004b). Moreover, in a very recent 120-d bed rest study, daily resistance exercise maintained knee extensor muscle size and strength (Shackelford *et al.* 2004). It is worth noting that in the present investigation only about 0.03% of the time spent in bed was dedicated to exercise for each muscle group. Still, this sparse stimulus was sufficient to maintain muscle size and specific function of the knee extensors.

Unlike the knee extensors, volume of the plantar flexor muscle group was not fully preserved by the resistance exercise program employed. It should be noted however, that the counteractive effect of resistance exercise was comparable, given that the 29% plantar flexor atrophy was reduced to 15% and the 18% knee extensor atrophy was abolished.

Yet, it cannot be excluded that inherent differences in responsiveness to resistance exercise among muscles impacted the counteracting effect on atrophy. For example, plantar flexors show only a modest increase in muscle size following chronic RE (Ferri *et al.* 2003, Weiss *et al.* 1988) and the soleus muscle exhibits lower rate of protein synthesis following an acute bout of resistance exercise compared with the VL muscle (Trappe *et al.* 2004b). These findings may in turn reflect that the plantar flexors contain relatively more type I fibres than the VL (Gollnick *et al.* 1974b, Johnson *et al.* 1973) and that these fibres display less hypertrophy than type II fibres in response to chronic resistance exercise (MacDougall 2003, Tesch and Karlsson 1985). In further support of this, the predominantly slow soleus muscle displayed greater atrophy than the more mixed gastrocnemius muscles, in response to bed rest and resistance exercise that evoked similar and maximal activation of both soleus and GM. Single-fibre data on the subjects examined here also revealed that resistance exercise had less of a protective effect on type I than II fibres of VL (paper IV, Trappe *et al.* 2004a). It can only be speculated that type I fibres and the plantar flexors which are more used in everyday locomotion also require more frequent low force activation for atrophy prevention. In fact, daily resistance exercise during 20 days bed rest maintained plantar flexor muscle size (Akima *et al.* 2003) and continuous but modest loading using sturdy rubber bands, i.e., the so-called “Penguin-suit”, during 60 days of bed rest preserved soleus fibre size (Ohira *et al.* 1999). Other regimens using frequent training failed to maintain plantar muscle size during 20 (Akima *et al.* 2000b) or 120 (Shackelford *et al.* 2004) days bed rest. Resistance exercise protocols employing training frequency similar to the present, prevented the very modest atrophy induced during 20 days lower limb unloading (Schulze *et al.* 2002). Interestingly, the increase in MVC was similar for the plantar flexors and knee extensors during training in confinement. Thus, it may be speculated that the presence of low-load stimuli due to daily postural activities, in addition to the resistance exercise program, aided in preventing plantar flexor deconditioning.

Despite that resistance exercise preserved MVC in the Supine Squat but not in the Calf Press, training specific muscle strength as well as training performance over the course of bed rest was maintained for both exercise modes. At first this appears somewhat surprising in light of the plantar flexor atrophy. Apparently, other adaptations must have compensated for the decrease in muscle size. It could be speculated that more effective use of the stretch-shortening cycle (Komi 2003) could have contributed to the maintained performance during dynamic Calf Press actions.

The plantar flexions, when performed during training, certainly call for substantial muscle lengthening. Changes in tendon stiffness (Reeves *et al.* 2005), may allow for greater usage of elastic energy, i.e. decreased hysteresis (Reeves *et al.* 2003). Such a response might be different between the plantar flexors and the knee extensors (Kubo *et al.* 2004a, b), and potentially contributing to the maintained force during the Calf Press exercise.

In contrast to training specific strength, peak torque assessed during unilateral knee extensions decreased in BRE, yet this effect was considerably less than in BR. This is not surprising since adaptations to resistance exercise are typically most evident in tasks performed during training (Morrissey *et al.* 1995, Rasch and Morehouse 1957, Rutherford 1988, Sale 1992, Sale and MacDougall 1981), and previous resistance exercise protocols have produced no or minute carry-over effect to strength assessed during isokinetic actions (Augustsson *et al.* 1998, Sleivert *et al.* 1995, Wilson and Murphy 1995). Moreover, bilateral training elicits less increase in unilateral compared with bilateral strength (Häkkinen *et al.* 1996, Taniguchi 1997), and multi-joint training produce less strength increase in single- than in multi-joint actions (Sale *et al.* 1992). The current data also accord with results from a 14 day bed rest study employing a leg press exercise countermeasure, which maintained task-specific performance but failed to offset the decrease in isokinetic knee extensor torque (Bamman and Caruso 2000). Given that muscle size of the knee extensors was maintained in the current study, failure to maintain isokinetic performance with the aid of resistance exercise could probably be attributed to neural mechanisms (Enoka 1997, Sale 2003), e.g., the different types of muscle actions require different coordination of synergists (Nardone and Schieppati 1988, Rutherford 1988, Rutherford and Jones 1986, Sale 1992) and/or different motor unit recruitment and activation patterns of particular muscles (Desmedt and Godaux 1977, 1979, Grimby and Hannerz 1977, Nardone *et al.* 1989). In contrast to isokinetic strength, performance or muscle activation in other non-training specific actions, i.e., sustained submaximal or ballistic maximal isometric actions, were essentially unaltered following bed rest with resistance exercise. This effect might be explained by the similarities between these two actions and the training modes, all being performed as bilateral, multi-joint actions in the Supine Squat position. Moreover, strength increases in response to chronic resistance exercise are most evident at joint angles (Lindh 1979) and angular velocities (Caiozzo *et al.* 1981, Kanehisa and Miyashita 1983, Narici *et al.* 1989) applied during training. In the present study isometric multi-joint tests were performed at hip- and knee joint angles that were within the range of that executed in the training mode. Moreover, a significant portion of the training action, i.e., the transition from an eccentric to a concentric action and the subsequent early acceleration, was performed at a very low angular velocity. Altogether (see Fig. 13), the current data clearly show that the protective effects of resistance exercise against strength loss during muscle unloading are more evident the closer to the training mode the action is. This would infer that counteracting compromised muscle function during unloading is not a matter of preventing atrophy only.

The finding of preserved RFD agrees with previous studies employing resistance exercise during short-term unloading (Bamman *et al.* 1998, Koryak 1998a). This effect seems logical in light of the increased RFD seen in response to resistance exercise (Aagaard *et al.* 2002, Häkkinen and Komi 1986, Thorstensson *et al.* 1976b) and may be attributed to the maintained muscle size but would also support unaltered motor unit

activation and recruitment (Grimby and Hannerz 1977, Miller *et al.* 1981). The fact that RFD was somewhat decreased immediately after bed rest but returned to baseline four days later, infer that important neural adaptations took place during the first days of reloading. The increased fatigue response was abolished as well by the resistance exercise regimen. This can be explained by maintained muscle volume but suggests also that motor unit recruitment during a sustained muscle action is unaltered. Altogether, these data suggest that the present resistance exercise protocol, calling for maximal activation, in addition to preserving muscle size, was effective in maintaining motor control during both maximal ballistic and submaximal sustained actions.

The shift in MHC composition of VL shown after BR, was attenuated by resistance exercise. Thus BRE induced no changes in MHC of IIa fibres or fibres containing IIX. This is not surprising since resistance exercise is known to favour a shift from IIX to IIa (cf. Tesch and Alkner 2003). However, a decrease in type I fibres and an increase in total hybrid fibres was present also in BRE. This is further evidence that the present training regimen more effectively protected type II than type I fibres. Given that only three subjects from each group were available for soleus biopsies, the finding of no MHC shift of soleus in BRE must be interpreted with caution. The trend was similar to the change manifest in VL. Hence it is not unlikely that a significant change may have occurred if samples had been obtained and analyzed from a larger subject pool.

IMPLICATIONS FOR SPACE AND CLINIC

The problem of muscle weakness experienced by astronauts following spaceflight was already identified during the Skylab era in the 60's (Nicogossian *et al.* 1994b, Rummel *et al.* 1975). Unfortunately, and though the issue has received considerable attention since then, as of today no effective countermeasures program, proven to successfully combat muscle atrophy and impaired function, is in effect. Among several proposed, some being very innovative yet somewhat fictitious (di Prampero 1994), means of countermeasures, e.g., electromyostimulation (Duvoisin *et al.* 1989, Mayr *et al.* 1999), insole pressure (Layne *et al.* 1998), lower body negative pressure with or without simultaneous exercise (Boda *et al.* 2000, Dupui *et al.* 1992, Lee *et al.* 1997, Louisy *et al.* 1990), centrifugation with or without simultaneous exercise (Clément and Pavy-Le Traon 2004, Kreitenberg *et al.* 1998), and dietary (Paddon-Jones *et al.* 2004, Paddon-Jones *et al.* 2005) or pharmacological (Caruso *et al.* 2005, Caruso *et al.* 2004) supplementation promoting skeletal muscle protein anabolism, perhaps resistance exercise remains the most attractive solution (Baldwin *et al.* 1996, Convertino 2002). Indeed, an apparatus providing reasonable resistance (Minigym) used by crew on the third Skylab mission was acknowledged as a potential countermeasure (Nicogossian *et al.* 1994a).

However, the vast majority of resistance exercise devices used in previous unloading studies (Akima *et al.* 2001, Akima *et al.* 2000b, Akima *et al.* 2003, Bamman *et al.* 1998, Bamman *et al.* 1997, Germain *et al.* 1995, Schulze *et al.* 2002) employed systems that are gravity dependent or not feasible in orbit for other reasons. The exercise device employed here can readily be used in space because it offers resistance independent of gravity and provides any other important feature typical of weight training apparatuses. It is also worth noting that bone loss, another severe consequence of unloading and spaceflight (LeBlanc *et al.* 2000b, Schneider *et al.* 1994, Vico *et al.*

2000), was prevented by chronic flywheel exercise performed by hind-limb suspended rats (Fluckey *et al.* 2002). Likewise, bone loss in the trainees of the current 90 day bed rest study, was attenuated and the magnitude of this effect was similar to that experienced by a subject group receiving medication against bone loss, i.e., Pamidronate[®] (Rittweger *et al.* 2005).

The present and the majority of previous unloading studies have focused on men, while data on women are scarce and not very comprehensive. While some indicate a somewhat greater atrophy in response to unloading (Shackelford *et al.* 2004, Suzuki *et al.* 1994), others do not (Funato *et al.* 1997, Tesch *et al.* 2003). This calls for more investigations on women. Actually, an ongoing study addresses the question how females respond to bed rest with or without concurrent exercise.

Albeit the current experiments were designed and aimed at simulating microgravity conditions by reducing or preventing weight-bearing, the findings here could also apply to the effects of unloading *per se* in e.g., populations characterized by a self-inflicted sedentary lifestyle, the elderly or in patients refraining from weight-bearing due to e.g., disease or previous trauma or surgery. It follows that the reported findings on effects of the current resistance exercise countermeasure paradigm can potentially be implemented to blunt any catabolic response and altered motor function in e.g., patients under convalescence. Due to the inherent features of the flywheel device, e.g., providing eccentric overload and calling for maximal effort (Berg and Tesch 1994, Tesch *et al.* 2004a), it might be beneficial also at 1g, in addition to traditional strength training regimens. In fact, unpublished and recently communicated data show that the elderly (Narici *et al.* 2005) and patients suffering from previous knee injury (Morrissey 2004) and neuromuscular disease (Zange, personal communication) benefit from flywheel training. Similarly, enhanced performance and reduced occurrence of hamstring injuries have been reported in elite soccer players adhering to a flywheel training programme (Askling *et al.* 2003). Collectively, the data generated from the current investigation and those of accompanying studies employing this novel exercise strategy, show that a very sparse exercise stimuli is capable of counteracting several of the negative consequences associated with a sedentary life style or lack of weight-bearing.

SUMMARY AND CONCLUSIONS

The present thesis evaluated the long-term effects of skeletal muscle unloading and the possible benefits of a resistance exercise paradigm. The main findings and conclusions are:

- Long-term unloading results in impaired function of lower limb muscles, mainly resulting from severe muscle atrophy and altered motor control
- The atrophy following short- and long-term unloading was greater for the plantar flexors compared to the knee extensors
- Alterations in motor control are suggested by decreased EMG amplitude during maximal and increased amplitude during submaximal actions
- The rates of atrophy and strength loss are markedly reduced with extended unloading
- A gravity-independent, multi-exercise flywheel resistance exercise device showed efficacy and feasibility when used during long-term confinement
- A flywheel device designed for bed rest use was found valid as a test and training device
- Resistance exercise performed every third day during long-term bed rest prevented atrophy of the knee extensors and attenuated the more severe plantar flexor atrophy
- Resistance exercise prevented loss in maximal strength, and also impairment in rate of force development and increased susceptibility to muscle fatigue
- Long-term unloading induced an increase in hybrid fibres and a transition towards faster MHC isoforms. This effect was attenuated by resistance exercise
- The slow-to-fast phenotype shift was not mirrored by *in vivo* muscle function alterations, e.g., force-velocity characteristics, rate of force development and fatigability
- The unaltered EMG amplitude during maximal and submaximal actions and unchanged rate of force development, suggest maintained motor control of the knee extensors in the exercise group
- The counteractive effects of resistance exercise on muscle function impairments were most pronounced in training specific tasks
- The present findings suggest that designing countermeasures against deteriorated muscle function extends beyond preserving muscle size only.

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