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A Study of the Efficacy of Therapeutic Electrical Stimulation in Minimizing Disuse Muscle Atrophy and Dysfunction of the Quadriceps Femoris Muscle Following Knee Surgery: A Model for Microgravity-Induced Disuse Atrophy

par

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Ce mémoire intitulé:

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présenté par

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Abstract

The purpose of this study was to evaluate the efficacy of therapeutic electrical stimulation (TES) designed to enhance quadriceps femoris muscle (QFM) recovery in patients following anterior cruciate ligament (ACL) reconstruction with a patellar tendon graft. Twenty-four patients were randomly assigned into two groups, an experimental group (n=12) and a control group (n=12). During the initial 12 postoperative weeks, all patients followed a standard rehabilitation program. In addition, the experimental group received treatment with TES for 6-8 hours, 5 nights per week. QFM isokinetic peak torque, work and mean power were measured in both limbs at 60°/sec. and 180°/sec. along with the neural activation (iEMG) and median power frequency (MED) of the vastus medialis and vastus lateralis muscles, preoperatively, and at 6 and 12 weeks postoperatively. All measurements were standardized as a percentage of the uninjured QFM. Results indicate that postoperatively, isokinetic peak torque, work and mean power were significantly reduced ($p < 0.01$) in the injured QFM when measured at either speed, both at 6 weeks and 12 weeks when compared to preoperative values. Vastus lateralis muscle iEMG activity was significantly reduced at 6 ($p < 0.01$) and 12 weeks ($p < 0.05$) postoperatively. However, no significant reductions in the vastus medialis iEMG activity were observed at either speed, both at 6 weeks and 12 weeks postoperatively. The MED had shifted to lower frequencies in both the vastus lateralis ($p < 0.01$) and vastus medialis ($p < 0.05$) muscles when measured at 6 weeks. However, by 12 weeks postoperatively, the MED of the vastus lateralis had returned to within preoperative values, while the vastus medialis shift in MED persisted ($p < 0.05$). No significant differences were observed between the control and experimental groups in terms of maximal isokinetic torque measurements and activation of the vastus lateralis and vastus medialis muscles throughout

the study period. In conclusion, TES in addition to rehabilitation, provided no extra benefit in terms of improving the recovery of the QFM during the early postoperative phase in patients who have undergone ACL reconstruction with a patellar tendon graft.

Résumé

Avec l'émergence des voyages spatiaux, plusieurs adaptations physiologiques ont été recensées en état d'apesanteur. L'apesanteur dans l'espace décroît l'activité et le chargement mécanique des muscles les plus importants pour déplacer et supporter le poids du corps, dans le but de maintenir une posture droite. Ces muscles, principalement les groupes musculaires des membres inférieurs, sont appelés muscles supporteurs du poids ; en réponse à une utilisation réduite, ils s'affaissent et dépérissent, ou s'atrophient. Les changements incluent une réduction de la grosseur du muscle [59, 60], la décomposition des protéines musculaires [61], la réduction de la force [69, 70] et de l'endurance [60] musculaires, de même que des changements dans les types de fibres présentes dans les muscles [61]. La faiblesse et le mauvais fonctionnement musculaires survenant suite à une exposition aux vols spatiaux réfèrent communément à l'atrophie musculaire fontionnelle induite par microgravité (l'atrophie musculaire fonctionnelle signifie une réduction du volume des muscles liée à l'inactivité).

Aucun de ces changements ne présente un problème pour les astronautes tant et aussi longtemps qu'ils n'effectuent que de légers travaux. Pour les astronautes, le problème devient critique lorsqu'ils reviennent sur terre et que les muscles affaiblis sont de nouveau soumis à la force de gravité intégrale. Dans une situation d'urgence, les individus aux muscles affaiblis seraient moins aptes à répondre rapidement ou à utiliser la force musculaire.

À présent, l'atrophie musculaire fonctionnelle des muscles des membres inférieurs induite par microgravité demeure un sérieux problème pour les astronautes qui poursuivent un vol spatial prolongé. Des contre-mesures comme l'exercice physique [91] peuvent aider au maintien de la force et des fonctions musculaires; toutefois, l'exercice seul est insuffisant

pour prévenir l'excès en perte musculaire. Ainsi, il est nécessaire de développer des solutions plus efficaces.

La présente recherche a été entreprise afin d'évaluer l'efficacité de la stimulation électrique thérapeutique (SET) pour minimiser l'atrophie musculaire fonctionnelle et le mauvais fonctionnement musculaire chez des patients ayant subi une reconstruction du ligament croisé antérieur (LCA). Le LCA est fréquemment lésé au cours de pratiques sportives. Une atrophie et un affaiblissement significatifs du groupe musculaire du quadriceps femoris (GMQF) survient suite à la reconstruction du LCA [131] et s'applique aux adaptations neuromusculaires survenant chez les astronautes suite à une exposition prolongée en état d'apesanteur.

L'objectif de cette recherche était d'entreprendre des expériences préliminaires afin de déterminer le paradigme de stimulation le plus efficace pour minimiser l'atrophie musculaire fonctionnelle des membres inférieurs induite par microgravité chez les astronautes. Le recours à des patients ayant subi une reconstruction du LCA fournit un modèle adéquat servant à étudier les traitements pouvant minimiser de tels déficits des fonctions musculaires.

Au total, 24 patients ont été assignés au hasard dans deux groupes, soit un groupe expérimental (n=12) ou un groupe contrôle (n=12), suite à la reconstruction du LCA grâce à une greffe du tendon patellaire. Durant les 12 semaines suivant l'opération, tous les patients ont suivi un programme standard de réhabilitation. De plus, le groupe expérimental a reçu un traitement par SET pendant six à huit heures, et ce cinq soirs par semaine. Le torque maximal isocinétique du GMQF, le travail et la puissance moyenne ont été mesurés dans les deux membres inférieurs à 60°/sec et à 180°/sec, en plus de l'activation neurale (iEMG) et de la fréquence de la puissance médiane (MED) des muscles vastus medialis et vastus lateralis, avant l'opération et à six et 12 semaines après l'opération. Toutes les mesures ont

été standardisées en pourcentage du GMQF sain. Les résultats indiquent qu'après l'opération, le torque maximal isocinétique, le travail et la puissance moyenne étaient significativement réduits ($p<0,01$) dans le GMQF lésé, et ce pour les deux vitesses, à six et à 12 semaines, comparativement aux valeurs préopératoires. L'activité iEMG du muscle vastus lateralis était significativement réduite à six ($p<0,01$) et à 12 semaines ($p<0,05$) suite à l'opération. Toutefois, aucune réduction significative de l'activité iEMG du vastus medialis n'a été observée, et ce pour les deux vitesses, à six et à 12 semaines après l'opération. Le MED est passé à des fréquences plus basses dans les muscles vastus lateralis ($p<0,01$) et vastus medialis ($p<0,05$) lorsque mesurée à six semaines. Cependant, 12 semaines après l'opération, le MED du vastus lateralis était revenu aux valeurs préopératoires, alors que le changement de MED du vastus medialis persistait ($p<0.05$). Aucune différence significative n'a été observée entre les groupes contrôle et expérimental en terme de mesures du torque maximal isocinétique et d'activation des muscles vastus lateralis et vastus medialis tout au long de l'étude. En conclusion, la SET combinée à la réhabilitation n'a pas fourni de bénéfice supplémentaire en terme d'amélioration de la récupération du GMQF au début de la phase postopératoire chez des patients ayant subi une reconstruction du LCA grâce à une greffe du tendon patellaire.

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List of Abbreviations

(+)	Increase
(-)	Decrease
AHP	After Hyperpolarization
AST	Angle-Specific Torque
DPA	Dual-photon absorptiometry
EM	Electron Microscope
EPP	Endplate Potential
FCSA	Fibre cross-sectional area
H/H	Hypokinesia/Hypodynamia
HDT	Head-down Tilt
iEMG	Integrated Electromyography
IF	Inflight
IFV	Interstitial Fluid Volume
LBNP	Lower body negative pressure
MCSA	Muscle cross-sectional area
MRI	Magnetic Resonance Imaging
MW/bw	Muscle weight to body weight ratio
NMJ	Neuromuscular junction
PF	Postflight
Po	Maximal Tetanic Tension
PT	Peak Torque
TDT	Terminal Deoxyneucleotidyl transferase
Vmax	Maximal Shortening Velocity
Vo	Maximal Shortening Velocity

Muscles

AE	Ankle Extensors
AF	Ankle Flexors
FT	Fast-Twitch
G	Gastrocnemius
KE	Knee Extensors
KF	Knee Flexors
LG	Lateral Gastrocnemius
MG	Medial Gastrocnemius
PL	Plantaris
QFM	Quadriceps Femoris Muscle
Sol	Soleus
ST	Slow-Twitch
SVL	Superficial Vastus Lateralis
TA	Tibialis Anterior
TB	Triceps Brachii

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Methodology

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For my mother, my brother Allen and Simon

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Many thanks go out to Phil whose dedicated approach to this research project and terrific sense of humor will never be forgotten. I would like to thank Simon Doucet for his invaluable technical assistance with the data collection, Dave Williams for his guidance and making this research possible, Leena and Pat for their support in keeping this project going, Eric Lenczner for his skilled contribution and willingness to learn new techniques, Francine for her generous assistance in recruiting patients and Jim Hanley for his help with all those statistics. Finally, I would like to thank Karen Pape for her superior knowledge and assistance with the stimulation procedures and Andrew Patrick for giving me the extra time to finish this thesis.

Muscular weakness and dysfunction arising from exposure to spaceflight is often termed "disuse atrophy" in much of the scientific literature. Spaceflight results in inadequate mechanical loading (hypodynamia) as generated by the weightless environment which in turn leads to decreased usage of the skeletal musculature. In combination with the removal of postural stimuli are reductions in motor activity (hypokinesia) of the antigravity musculature. Both mechanisms contribute to the atrophy seen in muscle exposed to prolonged spaceflight. The term "disuse", in this case, is unsuitable considering it refers to the discontinuance or cessation of muscle activity. Therefore, "underuse-related atrophy" is more appropriate terminology for the atrophic changes accompanying exposure to the hypodynamia and ensuing hypokinesia of spaceflight.

Underuse-related-atrophy is characterized by several neuromuscular adaptations that have been demonstrated in numerous ground-based investigations using models of mechanical unloading or simulated weightlessness, or both. In animals, the most commonly used models are joint immobilization and hindlimb suspension. In humans, joint immobilization, unilateral lower limb suspension (ULLS) and bedrest are frequently used to study the effects of unloading of the musculoskeletal system.

This review of the scientific literature will include the most recent findings using models of underuse-related-atrophy to characterize the neuromuscular adaptations to unloading and spaceflight. Comparisons between the results using different models, a list of possible mechanisms and proposed countermeasures will also be considered. Comprehensive tables reviewing the recent scientific literature relevant to each section are included. The tables include a brief description of the methodology,

significant results and comments on the major contributions of each study. Each section in turn, offers a brief description of the general findings presented in the corresponding table.

Part I: Structural and Functional Adaptations of Skeletal Muscle to Non-Weight-Bearing Activity in Animal Models

Atrophy of skeletal muscle during periods of unweighting/weightlessness are simply part of an adaptive process responding to a new physiological state. Chronic reduction of gravitational load on the hindlimb muscles having primarily an extensor/antigravity function such as the soleus (Sol), adductor longus (AL), medial gastrocnemius (MG), plantaris (PL), and vastus intermedius (VI) undergo atrophic responses to a greater extent than muscles having a primary flexor function like the tibialis anterior (TA) and extensor digitorum longus (EDL). Furthermore, fibre type composition of a muscle plays a role in determining the degree of response. Those muscles having a higher proportion of slow twitch oxidative fibres, as in the Sol, demonstrate a larger amount of atrophy in comparison to muscles with a lower proportion of this fibre type. Accompanying this atrophic response, a large portion of the slow twitch fibres in a postural muscle express fast myosin isoforms [1-3]. This transformation is largely manifested as hybrid fibers in which both slow (type I) and fast (type II) myosin heavy chain (MHC) are coexpressed in a given fibre [4].

Morphological Observations

Muscle fibre types are distinguished on the basis of their myofibrillar ATPase activity and in the older literature they are distinguished by their oxydative potential using selected key enzymes of anaerobic or aerobic

metabolism. Muscle fibres are classified as one of the following types: type I, type IIA and type IIB which correspond to approximately slow twitch oxidative (SO), fast twitch oxidative glycolytic (FOG), and fast twitch glycolitic (FG) in the old literature [5, 6]. Slow-twitch motor units contain SO fibres while fast-twitch motor units contain either FOG or FG fibres. Under normal physiological conditions, a given fibre is composed of myosin heavy chain (MHC) isoforms, where SO fibres contain slow (type I) MHC, FOG fibres contain fast (type IIa) MHC and FG fibres contain fast (type IIb) MHC. Postural muscles have a high proportion of SO fibres whereas fast muscles have a higher proportion of FG and FOG [5, 6].

Joint immobilization leads to profound atrophy of the muscles that function around the joint. Atrophy is generally defined as a decrease in muscle mass associated with a reduction in whole muscle cross-sectional area. The extent of atrophy is dependent on numerous factors namely: species, fibre type composition of muscle, duration of immobilization and position of joint fixation. All these factors have been extensively examined in animal models where direct measurements of muscle weights are obtained at the end of the study. A review of the recent literature regarding the morphological changes induced by models of mechanical unloading in animals is presented in Table I.

To summarize, the rate of atrophy is rapid following the onset of immobilization, (within the first 2-3 weeks), and then appears to proceed more gradually until a new steady-state is achieved [7, 8]. In terms of absolute muscle weight, a greater degree of atrophy arises in the Sol, a slow-twitch (ST) extensor muscle, compared to the EDL and the superficial region of the vastus lateralis (SVL), which happen to be fast-twitch (FT) muscles [7, 8].

Study	Ref #	Species	Model	Period	Method of Measurement	Results	Comments
Booth 1978	[173]	rat	casting	10d & 28d	Soleus and Gastrocnemius: MW, total protein content, CS activity	By 50th day of recovery all measurements had returned to control values in both Soleus and Gastrocnemius.	(1) Atrophic muscle can regrow entirely following immobilization for periods up to 28d. (2) Double the amount of time required for complete return to pre-atrophic muscle size.
Witzmann et al. 1982	[7]	rat	casting @ different lengths	1-42d	Soleus, EDL and SVL: MW;	MW: Sol -53%, EDL -34% and SVL -40%; Immobilization @ short length results in - Fiber length (sarcomere loss).	(1) Rate of atrophy is rapid following the onset of immobilization (within the first few weeks) but then proceeds more gradually until a new steady-state is achieved. (2) Slow postural muscles are more severely affected than fast muscles.
Spector et al. 1982	[174]	rat	pinned joints @ different lengths	4w	Soleus and MG: Architectural analysis: fibre length (FL), M-CSA, M-CSA=Volume/mean FL (volume=muscle weight/density) and fibre types (F-CSA)	Sol & MG IM in plantarflex.= shorter fibres w/ less sarc. reduced F-CSA. Chronic L of Sol & MG = longer fibres due to serial add. of sarc. TA, not as sensitive to weight loss in response to lengthening stimulus. Chronic L = proportional changes in F-CSA	(1) Muscle fibres in both slow and fast extensors fixed @ shortened lengths displayed reductions in FCSA and serial number of sarcomeres. (2) The opposite was true for the same muscle fixed @ lengthened position. (3) Fast flexor muscle was not as sensitive to fixation @ different lengths.
Appell 1986	[175]	mouse	casting (one hindlimb)	1, 4, 7, 11, 14, 18 d	TA: Morphometric and Structural Analysis using EM; FCSA.	7d: 35% atrophy, rate drops off w/ 50% atrophy by 18d. Red fibres more affected.Contra-controls atrophied 20% (18d). Pathologic alterations: No Striations, decomp. of myofibrils, disrupted mito, SR and central nuclei, leucocytes & satellite cells present.	(1) There is evidence of fibre regeneration in the TA muscle during immobilization.
Pierotti et al. 1991	[176]	cat	spinal isolation (SI)	24w	TA: FCSA	Following SI for 6 months: FCSA was reduced by 40% FR and 50% in FF fibres while the SO fibres were unchanged.	(1) Relative to the TA muscle, FCSA of the type IIB and type IIA fibres were reduced to similar extents while the type I were unaffected.
Jiang et al. 1991	[177]	cat	spinal isolation	24w	MG: FCSA	Almost a complete conversion of SO and FR to FF fibres; FCSA: -10%.	(1) Nearly all fibres were converted to type IIb with a reduction in mean FCSA in the MG muscle following 24w of SI.

Study	Ref #	Species	Model	Period	Method of Measurement	Results	Comments
Gardiner et al. 1992	[178]	rat	TTX of Sciatic nerve	14d	MG: MW, FCSA	MW: -48%; F-CSA: type II superficial (-68%) > type II deep (-61%) > type I (-48%).	(1) Complete neuromuscular inactivation of the MG by TTX results in atrophic responses that are more severe than HS and SF; including the pattern of susceptibility of the fibre types (II >I).
Musacchia et al. 1983	[10]	rat	Whole-body suspension (harness)	1w & 2w	Sol, G, Pl and EDL: MW absolute (mg) or relative (mg/100g body wt.).	MW losses: sol>gastroc=plantaris>EDL. Sol most sensitive, losing 35% & 45% of its weight during 1 & 2w respectively.	(1) The differing atrophic responses between the 4 hindlimb muscles coincides with those observed following exposure to microgravity.
Desplanches et al. 1987	[28]	rat	tail harness	5w	Sol and EDL: mATPase activity: fiber type distribution; FCSA;	MW: Sol -63%, EDL -22%; Distribution: -Type I w/+ in intermediate fibres: Ix (Sol) & IIx (EDL); Sol: FCSA type I-60%, type IIa -33%; EDL: no changes in FCSA.	(1) The Soleus exhibited a reduction in mean type I and IIa FCSA. (2) Hybrid (type I w/ type II MHC) fibres expressed at the expense of type I.
Stump et al. 1990	[12]	rat	HDS with single hindlimb supported (right limb).	14d	Sol and MG: Relative Mass (MW/100g bw)	MW/bw ratios for the HDS-L Sol, Pl and G were sig. < controls and HDS-R, HDS-R Sol being > control and HDS-R TA being < control.	(1) Support of the soleus during HS increased its mass beyond control values.
Riley et al. 1990	[13]	rat	tail harness	4, 7, 10 & 14d	Soleus: Relative MW, Histochemical and Ultrastructural analysis, F-CSA.	After 14d, type I & IIa fibres dec. in F-CSA by 63% and 47%. After 10d, 3% of fibres had segmental necrosis, all type IIa. By 13d, 30% fibres, mostly type I had central corelike lesions.	(1) The atrophic responses and pathological changes observed in both fibre types may be a consequence of the altered use and compromised blood flow of the soleus muscle during hindlimb suspension.
McDonald and Fitts 1993	[16]	rat	tail harness	1, 2 or 3 wk	Single Sol Fibres: fibre diameter, ATPase activity, relative content of slow and fast myosin using SDS-PAGE.	ATPase activity showed a significant increase between 1 & 3 wk, associated w/ a greater % of type IIa fibres and both were highly correlated.	(1) Soleus muscle exhibited a steady decline in MW/bw ratio, FCSA while ATPase activity gradually increased. (2) Over the 3 week period, the effects of unloading were more dramatic during the first and last week.

Study	Ref #	Species	Model	Period	Method of Measurement	Results	Comments
Taillandier et al. 1993	[17]	rat	tail harness	3 wk	**Soleus:** fiber-type distribution and F-CSA; Rats fed a high-HP (30%) or medium-MP (15%) protein diet.	**HP** diet had no benefit in preventing atrophy; 65% dec. in **FCSA. HP** diet maintained higher % of type I in HS, whereas, HS-MP rats had -42% in type IIa & hybrids.	(1) Both high and moderate-protein intake had no effect on the atrophic responses induced by HS. (2)During HS, the intake of a high-protein diet partially conserved the fiber-type distribution in the Soleus.
Allen et al. 1997	[179]	rat	tail harness	14d	**Soleus:** TDT labelling as an indicator of apoptosis for analysis of myonuclei.	Sig. inc. in number of **TDT+** muscle nuclei w/ a progessive inc. up to 7d then plateau. Sig inc. in number of fibres containing morphologically abnormal myonuclei.	(1) The removal of existing myonuclei by apoptosis may be moderately accountable for the decline in myonuclei accompanying HS.
Musacchia et al. 1990	[46]	rat	whole-body w/ HDT	7d & 14d	**Soleus and EDL:** MW (absolute) and F-CSA	**Soleus:** absolute mass -30% @14d; -STand FT CSA@7, 14d w/ greater losses in FT @ 14d; + ST density @ 7&14d; **EDL:** no change in mass @14d; +ST FCSA@14d; -FT fibre density@7d w/recovery@14d; 7d WBS recovered by 7d.	(1) Atrophic responses accompany HS in both ST and FT fibres of the Soleus, but are more pronounced in the FT fibres at 14 days.
Jiang et al. 1992	[30]	rat	tail harness	2w	**Single fibres of MG (FT ext) and TA (FT flex):** FCSA; Fibers catagorized on basis of immunohistochemical rxn.	**MG:** +%of only hybrid fibres; **TA:** + 14% in FT CSA; **Both MG and TA:** Mean fibre size of any type was unaffected in either muscle.	(1) Generally, the responses were more pronounced in the MG than the TA muscle. (2) Mean fiber size of any type was unaffected in either muscle.
Musacchia et al. 1992	[180]	rat	tail harness	14d	**Vastus Medialis:** fiber density (fibre/mm2) , FCSA.	**Fibre density** in both homogeneous and mixed portions were sig. < corresponding regions of spaceflight muscles;	(1) There is an absence of type II fiber change in the VM following HS.
Ohira et al. 1992	[15]	rat	tail harness	14d	**Soleus fibers:** Immunohistochemical staining for MHC's; F-CSA.	**Hybrid fibres**+7% @ the expense of ST fibres; **Mass** -34%; Mean ST CSA -44%;	(1) ATPase activity was unaffected by HS. (2) The ATPase activity of hybrid fibres is comparable to that of type I fibres reflecting a dissociation between myosin isofrom and ATPase activity within these
Riley et al. 1992	[14]	rat	tail harness	14d	**Adductor Longus** (ST anti-G muscle): MW, F-CSA, Ultrastructural analysis.	**MW:** -40%; ST-CSA decreased > FT fibres; hybrid fibres; ECC-like lesions were not as severe as in spaceflight because they were not subjected to the reentry and landing forces.	(1) Atrophic responses accompany HS in both ST and FT fibres of the AL muscle, but are more severe in the ST fibres

Study	Ref #	Species	Model	Period	Method of Measurement	Results	Comments
Henriksen et al. 1993	[9]	rat	tail harness	1-6d	Soleus and EDL: Relative mass, IFV assesed by inulin space.	Sol: @ 1d no change in weight; @ 3d -14%, @ 6d -32%; IFV: @ 3d +35%, @6d +53%; EDL: @6d +5% due to stretch; IFV: unaltered. Both: Absolute IFV (relative to BW) unchanged.	(1) Soleus muscle exhibited a negative correlation between muscle weight and IFV.
Talmadge et al. 1996	[4]	rat	tail harness	14d	Soleus: SDS page analysis and Immunohistochemistry of MHC isoforms.	De novo synthesis of type IIx (6%) MHC but no significant change in type I and IIa proportions after HS and SF. Mutiple MHC fibres +26% in HS.	(1) A conversion sequence of MHC isoform expression from type I - IIa - IIx - IIb emerges in response to unloading by HS. (2) The transition rate can be quite rapid and consequently fibers with multiple MHC isofroms can result at a given time.

Table I: Muscle morphological observations following periods of non-weight bearing activity in animals

Immobilization, spinal isolation, cord transection and tetrodotoxin (TTX) treatment are all models designed to simulate the effects of weightlessness. While these models yield atrophic responses in the skeletal musculature, they are limited as simulations of weightlessness because they utilize restriction in limb mobility, unnatural limb positioning at different lengths or removal of electrical neural input. In contrast, suspension models including whole-body and tail suspension more realistically simulate the combination of hypokinesia and hypodynamia experienced during spaceflight. Unlike limb casting or denervation, suspension models permit a full range of voluntary contractions and the freedom to maintain normal resting lengths.

Hindlimb suspension (HS) is commonly used to simulate the combined hypokinesia and hypodynamia experienced during weightlessness. In this model, the hindlimbs are not permitted to touch the ground and the gravitational force of the body weight is removed from the hindlimbs. Comparable with immobilization, data from HS studies show that atrophic responses of the hindlimb musculature appear early, but to varying degrees among the different muscles where ST extensor muscles are primarily affected over FT muscles.

Briefly, as early as 3 days, a significant (14%) loss in relative Sol mass (mg/100g body wt) is observed [9] and the amount of atrophy continues to increase with a final 27-29% reduction in Sol mass after 7 days of HS [10, 11]. Atrophic responses appear to slow down and by the end of the second week, Sol mass is comparable to that seen after one week of HS [12]. In contrast, the MG, a FT extensor, exhibits a lesser response with a 10-19% drop in relative mass after 1-2 weeks of HS [10-12]. In comparison to extensors, flexor muscles appear to be more resistant to the effects of HS where little or no significant change in muscle mass is observed [9, 10, 12].

Cross-sectional area (CSA) measurements of individual muscle fibres offer a direct and fairly accurate method for estimating the amount of atrophy. Both type I and II fibres demonstrate significant atrophy as measured by fibre cross-sectional area (FCSA). However, type I fibres, predominently found in ST muscles, tend to be more atrophied in comparison to the type II fibre population [13, 14]. For example, both the Sol and AL muscles exhibited reductions in mean FCSA of both fibre types but the response was greater in the type I fibre population after 2 weeks of HS [13, 14].

Myosin isoforms (MHC) of individual fibres, categorized on the basis of immunohistochemical reaction, have been classified as slow type I and fast type IIa, IIx and IIb. Phenotypic MHC expression is modulated by the amount of gravitational loading where, in the absence of weight bearing activity, the incidence of type IIx MHC expression (a transitory fast MHC isoform) increases in the preexisting type I and IIa fibres of the soleus muscle [4].

Based on myosin isoform (MHC) expression, significant alterations in fibre type distribution following HS have primarily been observed in the Sol muscle, composed predominantly of type I MHC in the rat. To briefly summarize, evidence suggests that the Sol muscle demonstrates a significant shift in its MHC profile from predominantly slow (type I) MHC towards a fast (type II) MHC expression [2]. After 2 weeks of HS, a 7% decrease in fibres expressing type I MHC was counterbalanced with a similar increase in fibres expressing both type I and II MHCs, commonly referred to as hybrid fibres [15]. In addition, Talmadge et al. [4] observed a 26% increase in fibres expressing multiple MHC isoforms following HS, suggesting a transition sequence of MHC isoform expression from type I→IIa→IIx→IIb may occur in the Sol in response to unloading by HS.

Therefore, numerous investigations examining the effects of unloading on fibre type distribution within the rat soleus have consistently reported

increases in the percentage of fibres expressing type II phenotypes and an augmented presence of hybrid fibres at the expense of the type I fibre population, with the magnitude of the response being dependent on the duration of HS [4, 15-19].

Protein Composition

The reported reductions in muscle mass and FCSA are accompanied by alterations in the protein composition of the unweighted muscles. Generally speaking, muscles are composed of 75% water and 20% protein, with 60% of the total protein content attributed to the myofibrillar proteins. A review of the recent data on protein composition during models of unloading in the rat are presented in Table II.

In general, marked reductions in total protein content (mg/muscle) following periods of unweighting are observed in ST skeletal muscle to a greater extent than FT skeletal muscle [20, 21]; thus resembling the changes in muscle mass. For example, corresponding with the observed reductions in absolute Sol muscle mass, Savolainen [20] reported losses of 23% and 43% in total protein content per Sol muscle pair after 1 week and 3 weeks of immobilization, respectively [20]. Likewise, at 10 days of HS, a 31% loss in absolute Sol mass was accompanied by a matching reduction in total protein content [22].

Owing to the fact that proportionally, myofibrillar proteins make up the largest fraction of total muscle protein content, observed decrements in overall content are reflected in the preferential loss of myofibrillar proteins. For example, Linderman et al. [21] noted a 26% decrement in the myofibrillar protein content of the gastrocnemius muscle following 5 days of HS, whereas no observed losses were reported in the Sol. In contrast, profound losses of

Study	Ref #	Species	Model	Period	Method of Measurement	Results	Comments
Musacchia et al. 1983	[20]	rat	Whole-body suspension (harness)	1w & 2w	Sol, G, Pl and EDL: MW absolute (mg) or relative (mg/100g body wt.), Urine analysis: urea and NH₃ and 3-methylhistidine (3MH)	MW losses: sol>gastroc=plantaris>EDL. Sol most sensitive, losing 35% & 45% of its weight during 1 & 2w respectively. Products of muscle metabolism: urea, NH3 and 3MH increased during suspension are sig. reduced to control levels during recovery.	(1) The differing atrophic responses between the 4 hindlimb muscles coincides with those observed following exposure to microgravity. (2) Elevated 3-MH in the urine confirms the loss of myofibrillar proteins.
Savolainen 1987	[2]	rat	Casting @ 90o	1w & 3w	Gastroc, Sol and TA: MW, Protein content, Proteolytic enzyme activities: Cathepsin D (CD), β-glucuronidase (β-g), alkaline protease (AP), acid autolysis (AA).	MW: @ 1w : Sol -27%, G -31%, TA -19% @ 3w; Sol -39%, G -42%, Ta -35%; Protein Content: @ 1w Sol -26%, G -33%, Ta -18%; @ 3w Sol -43%, G -44%, TA -31%; Enzymes: CD Normally Sol>G and TA, Sol nc, G +, TA +; β-g: nc; AP: G+@ 1w, TA + @ 3w; AA: G+ and TA+.	(1) Immobilized muscles showed a clear and progressive decline in total protein content. (2) During immobilization, proteolytic and AA activities increase reflecting the excessive protein breakdown within the muscles. (3) The activity of CD differs between dorsiflexors and plantarflexors following immobilization.
Thomason et al. 1987	[83]	rat	tail harness	4d - 56d	Soleus: MW; Myofibrillar ATPase activity;myofibril protein content; MHC isoform expression.	Myofibril ATPase activity: no change; Protein content: -80%; Slow myosin (SM) content: -84%; Myosin isoform expression: -SM with equal +fast myosin (FM) isoform. Loss of myofibrillar protein occured to a greater extent than soleus mass loss.	(1) A significant shift in the myosin isoform profile from predominently slow myosin towards fast myosin expression. (2) This shift was mediated by the net degredation of SM isoforms and apparent de novo synthesis of the fast isoforms.
Thomason et al. 1989	[185]	rat	hindlimb unweighting	5h & 1w	Soleus: Protein synthesis rate : [³H] Leu and estimate the time course of changes in myofibril synthesis and degradation rates. Measured β-myosin heavy-chain mRNA concentration to investigate the role of transcriptional regulation.	Sol exibits a dec. in total mixed & myofibril protein synthesis rates within 5 hr of HS (ns). By 7d, Sol muscle exhibits a significant decrease in both total mixed (46%) & myofibril protein synthesis rates (59%). No decrease in [β-myosin HC mRNA] @7d.	(1) A rapid but ns. reduction in the synthesis rate of myofibril protein is observed resulting in a net loss of myofibril protein in the soleus muscle. (2) This decrease in the synthesis rate persists over the next 7 days leading to significant losses in myofibril protein. (3) This decline in the rate of myofibrillar synthesis does not appear to be related to transcriptional regulation.

Study	Ref #	Species	Model	Period	Method of Measurement	Results	Comments
Steffen et al. 1990	[17]	rat (adult & juvenile)	Whole body w/ HDT	1, 7, or 14d	**Sol and Gastroc.:** MW, Protein/DNA ratio (index of muscle cell size), **RNA/DNA** ratio (index of protein synthetic capacity).	Adult & **juvenile** differential muscle responses comparable but juvenile **more responsive to a short period of disuse**(within 7 days) than adults: juvenile Sol, G weights (within 7d) and a-actin mRNA dec more rapidly (within 1 day) than adults (within 7d).	(1) During the initial week of HS, juveniles exhibited consideraby greater losses in muscle weight and decrements in a-acting mRNA in comparison to adults. (2) The following week, adult and juvenile responses were similar.
Taillandier et al. 1993	[22]	rat	tail harness	3 wk	**Soleus: K_s;** protein synthesis rate, total protein content, fiber-type distribution and F-CSA, T_3 circulation levels of Soleus. Rats fed a high-HP (30%) or medium-MP (15%) protein diet.	HS reduced **Ks** (33%) in **MP** but not HP. **HP** diet had no benefit in preventing atrophy; 65% dec. in **FCSA.** HP diet maintained higher % of type I in HS, whereas, HS-MP rats had -42% in type I w/ + in type IIa & hybrids; HP induced decrease in T3 level.	(1) Both high and moderate-protein intake had no effect on the atrophic responses induced by HS. (2) During HS, the intake of a high-protein diet circumvented the reduction in Ks and partially conserved the fiber-type distribution in the Soleus. (3) A possible mechanism for the high-protein effects may include an hormonal response involving thyroid hormones.
Kirby et al. 1992	[36]	rat	tail harness	14d	**Soleus:** MW and Protein content and concentration (noncollagenous).	MW: -31%; **Protein Content:** -29.5% **Protein Concentration:** unaffected in Sol.	(1) HS induced significant decrements in Sol noncollagenous protein content (mg/muscle), quantatively similar to the observed losses in absolute Sol muscle mass.
Tischler et al. 1993	[21]	rat (juvenile)	tail harness	7d	**Sol, PL, MG, and EDL:** Relative MW, protein content and insulin sensitivity.	MW/100g body wt.: Sol -33%, MG -9%, TA and EDL ns; **Protein Content:** Sol -23%, PL and MG showed a net accumulation of protein; **Insulin response on Glucose uptake:** Sol +71%. Also, insulin increased uptake 2.7 -fold in Sol relative to 1.6 in controls.	(1) HS is suitable for mimicking the effects of weightlessness on rapidly growing muscle of juvenile rats.
Linderman et al. 1994		rat	tail harness	5d	**Sol and Gastroc:** MW, Mixed and Myofibrillar protein content (mg/muscle) and synthesis (fraction/day).	MW: Sol -31%, G -20%; **Protein content:** _Mixed_ Sol -21%, G -16%; _Myofibrillar_ unaffected in Sol, G -26% ; **Protein Synthesis:** _myofibrillar_ -51% and _mixed_ -53% in Gastroc, Sol insufficient tissue for analysis..	(1) Similar to muscle mass, mixed and myofibrillar protein contents rapidly decreased following HS. (2) Myofibrillar protein content is differentially affected by HS in the Soleus and Gastrocnemius.

Table II: Muscle protein composition following periods of non-weight bearing activity in animals

approximately 75-85% in myofibrillar protein content per Sol muscle pair have been demonstrated after 4-8 weeks of hindlimb unweighting [1, 2]. Most of this loss in the rat Sol appears to be attributed to the preferential degradation of slow myosin isoform [2].

Therefore, these data indicate that the major impact of suspension is likely to be the preferential loss of the myofibrillar proteins, which directly coincides with the absolute reductions in muscle mass, with the magnitude of the response related to the amount of slow myosin content within the muscle.

Functional Properties

In animal models, *in situ* muscle tension generating capacity is evaluated by measuring the maximal tetanic tension (P_o) in grams. Specific tension (g/cm^2) is a measure of P_o per unit whole muscle CSA commonly used as a more accurate measure of intrinsic muscle force since the tension exerted is measured independent of size. Both indices of muscle force production have been reported to decline in the rat soleus following periods of immobilization [7, 23] and hindlimb suspension [16, 19, 24, 25]. For a more comprehensive review of the data, please refer to Table III.

Briefly, Simard et al. [23] reported that 4 weeks of immobilization at a shortened length can induce significant changes in the histochemical profiles and reduction in specific tension of the Sol and MG muscles, but speed-related properties remained unaffected. Conversely, immobilization at a lengthened position can delay the onset of these responses [23].

Twitch time course is repeatedly reduced in immobilized soleus muscle [7, 8, 23], the force-frequency relationship reportedly shifts to the right [25] and the maximal shortening velocity (Vmax) is either unchanged

Study	Ref #	Species	Model	Period	Method of Measurement	Results	Comments
Witzmann et al. 1982	[7]	rat	casting @ different lengths	1-42d	**Soleus, EDL and SVL:** MW; Isometric contractile properties (Po); Vmax, Length tension relations.	MW: Sol -53%, EDL -34% and SVL -40%; **L-T relation:** shifted to the left (- extensibility) in Sol; **Po:** Sol -53%, EDL -28% and SVL unaltered; **Vmax:** Sol +67%, EDL +35%, and SVL +20%. **Immobilization @ short length** results in -Fiber length (sarcomere loss).	(1) Rate of atrophy is rapid following the onset of immobilization (within the first few weeks) but then proceeds more gradually until a new steady-state is achieved. (2) Slow postural muscles are more severely affected than fast muscles.
Witzmann et al. 1982	[8]	rat	casting	6w	**Soleus and EDL:** MW; contraction time (CT), Half-relaxation time (1/2RT), Peak twitch tension (g/cm^2) and P_o	SOL lost a greater % of wet weight than EDL and SVL but recovery rate was greater and full recovery was faster in the Sol **(due to high protein turnover rates).** P_o fell sig. in SOL and both SOL and EDL recovered by 28d. Vmax inc. in all muscles.	(1) Rate of atrophy initially begins rapidly, within the first 2-3 weeks and then proceeds at a slower pace. (2) Greater degree of atrophy arises in slow postural muscles compared to faster contracting muscles; (type I>II). (3) Full recovery of normal contractile function following immobilization. (4) Rate of recovery is contingent upon the fibre type composition of the affected muscle, whereby, slower muscles more rapidly recover than faster muscles.
Simard et al. 1982	[23]	rat	pinned joints @ different lengths	4w	**Sol, MG and TA:** Speed-related properties: CT, 1/2RT, Vmax and **Tension Properties:** Pt and Po	CT and **1/2RT** were shorter than normal for the SOL fixed @ N. **Vmax/ # sarc.** was not affected in any muscle. No change in **Po/MW** of SOL, MG and TA @ N. **Po/CSA** of SOL & MG was maintained and reduced @ L and S respectively. Po/CSA TA was reduced @ L and S.	(1) Immobilization @ shortened and neutral lengths can induce changes in the histochemical profiles and contractile properties of slow muscle, but the shortening properties remain unaffected. (2) Immobilization @ lengthened position can delay the onset of atrophic responses in soleus and medial gastrocnemius.
Gardiner et al. 1992	[178]	rat	TTX of Sciatic nerve	14d	MG: Po, Fatigue Index	**Absolute Po:** -70%, **SDH activity:** type I: -25%, type II: -37%.	(1) Complete neuromuscular inactivation of the MG by TTX results in atrophic responses that are more severe than HS and SF; including the pattern of susceptibility of the fibre types (II > I).

Study	Ref #	Species	Model	Period	Method of Measurement	Results	Comments
Fell et al. 1985	[11]	rat	whole-body	1w	**Sol and Gastroc.: Fatiguability:** (Train stimulations 45/min for 16 min); **Contractile properties:** (twitch and tetanic) of S and G	Faster rate of fatigue in G and fatigue resistance was preserved in S of H/H suspended rats. Contractile properties (tensions per gram of wet muscle: g/g) in G and S were unaffected.	(1) A greater rate of fatigue was observed in the G, whereas the Sol maintained its resistance to fatigue following 1w of suspension. (2) The contractile properties of both Sol and G were unaffected.
McDonald et al. 1992	[26]	rat	tail harness	15d	**Soleus:** Relative blood flow and Fatiguability during 10 min of stimulation @100Hz	After 15 d HS, Sol had a reduced resistence to fatigue during intense contractile activity; however, soleus blood flow per 100g muscle was not altered.	(1) The soleus muscle demonstrated an increase in fatiguability which does not appear to be related to the blood flow to the muscle.
McDonald and Fitts 1993	[16]	rat	tail harness	1, 2 or 3wk	**Single Sol Fibres:** maximal velocity of shortening (**Vmax** or Vo), **ATPase activity**, relative content of slow and fast myosin using SDS-PAGE.	**Po** was sig. reduced after 1wk of HS (18%), but showed no further change in 2-3 wk of HS. **Vmax** and **ATPase activity** showed a significant increase between 1 & 3 wk, were ass. w/ a greater % of type IIa fibres and both were highly correlated.	(1) Soleus muscle exhibited a steady decline in absolute force while the maximal shortening velocity and ATPase activity gradually increased. (2) Over the 3 week period, the effects of unloading were more dramatic during the first and last week.
McDonald et al. 1994	[19]	rat	tail harness	1, 2 or 3 wk	**Single Soleus fibre** Peak force (N) specific tension (kN/m²), **Vo** and **force-velocity characteristics**, myosin analysis and fiber-type distribution using SDS-PAGE.	HS shifted **F-V curve** such that **Vo** inc. for a given fibre load. **Absolute power output** dec sig after 1wk; continued decline thru 2wk but reached new lower steady state by 3wk. **FT** % in Sol inc. to 26% by 3 wk, but most fibres with inc Vo contained ST myosin.	(1) Fibres demonstrated a progressive decline in absolute power output until a plateau was reached by the 3rd week. (2) The force-velocity relation shifted upwards. (3) A greater percentage of ST fibres featured an increase in Vo without altering the MHC profile.
McDonald and Fitts 1995	[24]	rat	tail harness	1, 2 or 3 wk	**Single Soleus fibre:** fibre diameter, Po, elastic modulus or stiffness (Eo), stiffness to CSA ratio (**index of # of attached cross bridges**), force pCa and stiffness-pCA relationships.	HS resulted dec.absolute force, **Po** and rightward shift in **force-pCa** and **stiffness-pCa** relationships of single fibres. **Po/Eo** was increased because HS induces decline in both parameters but the effect on stiffness (Eo) is much greater.	(1) The loss of contractile proteins induced by HS causes the myofibrillar lattice spacing to increase and, in turn, a reduction in the number of myofibrillar cross-bridges per fiber area and consequently reduces the fiber Po and Eo.

Study	Ref #	Species	Model	Period	Method of Measurement	Results	Comments
Leterme and Falempin 1996	[25]	rat	tail harness	14d	**Soleus:** MW, Po, Pt, TTP, HRT and Fatigue index (FI), sag properties.	**Reorganization** of Sol m.u. profile: 50% of m.u. pop. presented sag, vs 15% in control - 26% Sl-type. **Pt** drop in FT m.u.; **Po** fell in both ST & FT m.u. **Tension-Hz curve** shifted to right: FT mu had lower tensions @ higher Hz. ST had inc. tension @ lower Hz.	(1) The presence of SAG in the unloaded soleus came from the slow motor units whose altered speed-related properties demonstrated faster characteristics.

Table III: Muscle contractile properties following periods of non-weight bearing activity in animals

[23] or increased [7, 16, 19] following periods of immobilization and HS. Vmax is positively correlated with fibre myofibrillar ATPase activity and an increase in one parameter often coincides with an increase in the other [16]. Furthermore, there is evidence for a possible reorganization of the Sol motor unit profile with the presence of *sag* (normally a feature of FT motor units) in 50% of the motor unit population of primarily ST motor units [25]. These observations are consistent with the unloaded Sol exhibiting a shift in its myosin profile towards fast myosin expression.

Single Sol fibres demonstrate a progressive decline in absolute P_o until a new steady-state is reached by the third week of suspension [16, 19]. McDonald et al. [24] suggested that the decrease in Sol fibre P_o might result from the reduction in the number of myofibrillar cross-bridges per unit fibre area due to the increased myofibrillar lattice spacing caused by the loss of contractile proteins induced by HS.

The relative decrease in force production over time is commonly used to measure the fatigue characteristics of a muscle. In the Sol, the ability to maintain its resistance to fatigue appears to be influenced by the length of HS. For example, Fell et al. [11] reported that the Sol maintained its resistance to fatigue following one week of HS. However, after 15 days of HS, an increase in fatiguability was demonstrated in the Sol muscle during contractile activity for 10 min. at a stimulation frequency of 100 Hz [26].

Metabolic Properties

Indices of oxidative capacity include the measurement of total enzyme activities related to aerobic metabolism such as cytrate synthase (CS) and succinate dehydrogenase (SDH) activity as well as muscle capillarization (capillaries/fibre). Similarly, indicators of anaerobic metabolism include

measurement of glycolytic enzyme activities such as α-glycerophosphate dehydrogenase (GPD) and phophofructokinase (PFK). Due to the altered energy demands of skeletal muscle during periods of unloading, HS has been shown to induce alterations in the metabolic profiles of ST [12, 15, 27, 28] and FT [27, 29, 30] muscles. In general, the oxidative potential of the hindlimb musculature following suspension appears to decrease in the extensors with no changes observed in the flexors. In contrast, both extensors and flexors demonstrate an augmented glycolytic potential. Metabolic studies on selected muscles of the rat during periods of unweighting are presented in Table IV.

In summary, the oxidative potential of the Sol has been reported to decrease with reductions in mean total CS activity (μmol/g/min) [12, 27, 28] and mean total SDH activity (mean optical density (OD)/min x FCSA) [15, 31, 32] and capillarity [28] following HS. Furthermore, in some cases, Sol muscle fibres have emerged with an enzyme profile that has shifted slightly toward an elevated glycolytic capacity [15, 29]. The observed responses in Sol metabolic profile are consistent with the upregulation of fast MHCs, overall exhibiting a trend towards a faster and more fatiguable muscle. Conversely, both EDL and TA muscle fibres exhibited no modifications in mean total CS [28] and SDH [30] activities, respectively, whereas PFK and GPD activities were increased in muscle fibres of the TA following HS [29].

Neural Adaptations

Recent studies investigating the neuromuscular adaptations related to neural activation that arise in rat hindlimb musculature during periods of unloading are reviewed in Table V. Briefly, electromyogaphy (EMG) activity of the rat hindlimb musculature immobilized at shortened and neutral lengths

Study	Ref #	Species	Model	Period	Method of Measurement	Results	Comments
Jiang et al. 1991	[177]	cat	spinal isolation	24w	MG: SDH activity, GPD activity, FCSA	Almost a complete conversion of SO and FR to FF fibres. **Mean SDH activity:** -70%; **Mean GPD activity:** +120%; **FCSA:** -10%.	(1) Nearly all fibres were converted to type IIb with a reduction in mean FCSA in the MG muscle following 24w of SI. (2) Mean oxidative and glycolytic potential were decreased and increased respectively.
Fell et al. 1985	[27]	rat	Whole body	1w	**Sol and Gastroc:** Oxidative capacity: CS activity & [cytochrome c] **Insulin sensitivity:** Glucose uptake rates.	CS activity: -17% in Sol and -23% in G from H/H rats. **[cyt c]:** -29% dec. in G . Rates of glucose uptake were lower in muscles of H/H rats but were found to have increased [glycogen].	(1) Reduction in the oxidative capacity of the gastroc. > in the soleus as demonstrated by the diminished activity of CS in these muscles. (2) A greater storage of glycogen was observed and might possibly be related to the insulin insensitivity by negative feedback.
Desplanches et al. 1987	[28]	rat	tail harness	5w	**Sol and EDL:** VO2max; mATPase activity: fiber type distribution; FCSA; Capillarity (caps/fibres); CS activity.	**VO2max:** -19%; **MW:** Sol -63%, EDL -22%; **Distribution:** -Type I w/+ in intermediate fibres: Ix (Sol) & IIx (EDL); **Sol: FCSA** type I-60%, type IIa -33%; - capillarity > CS and 3-HAD activities; **EDL:** no changes in FCSA, Caps, enzymes.	(1) The Soleus exhibited a reduction in mean type I and IIa FCSA. (2) Capillarity is reduced and correlated with a decrease in oxidative capacity. (3) Hybrid (type I w/ type II MHC) fibres expressed at the expense of type I.
Stump et al. 1990	[12]	rat	HDS with single hindlimb supported (right limb).	14d	**Sol and MG:** Glycogen concentration and CS activity.	Both HDS Sol CS activity were < than control. HDS-L Sol [glycogen] was sig. > both HDS-R and control.	(1) Support of the soleus during HS paralleled the unsupported soleus and exhibited a loss in CS activity, (2) HS muscles develop high concentrations of glycogen.
Musacchia, Steffen et al. 1990	[46]	rat	whole-body w/ HDT	7d & 14d	**Soleus and EDL:** Capillary density (caps/mm²) and F-CSA	**Soleus:** -ST and FT CSA@7, 14d w/ greater losses in FT @ 14d; + ST density @ 7&14d;+capillary density@7&14d. **EDL:** +ST FCSA@14d; -FT fibre density@7d w/recovery@14d; +cap density @14d. 7d WBS recovered by 7d.	(1) Atrophic responses accompany HS in both ST and FT fibres of the Soleus, but are more pronounced in the FT fibres at 14 days. (2) The observed increase in capillary density may be due in part to the reduction in CSA of fibres, therefore, increasing the number of fibres per unit area.

Study	Ref #	Species	Model	Period	Method of Measurement	Results	Comments
Chi et al. 1992	[29]	rat	tail-harness	2w	Soleus and TA: Metabolic Enzyme Assays: PFK, pyruvate kinase, GPD and Hexokinase being usually more abundant in type II (GLYCOLYTIC); MDH and KACAT are OXIDATIVE.	Soleus fibres: pyruvate kinase, GPD and PFK were (ns) elevated; no change in KACAT; hexokinase was greatly increased. TA fibres: hexokinase +83%; PFK, GPD were + in type IIa; MDH no change in any fibre type.	(1) Following suspension, the soleus fibres emerge with an enzyme profile that has shifted slightly toward an elevated glycolytic capacity.
Jiang et al. 1992	[30]	rat	tail harness	2w	Single fibres of MG (FT ext) and TA (FT flex): FCSA, SDH, GPD, and ATPase activites; Fibers catagorized on basis of immunohistochemical rxn.	MG: -SDH and total SDH activities (OD/min x CSA (um2)) of FT; +GPD/SDH ratio in FT. TA: +GPD & total GPD activities in FT.	(1) Generally, the responses were more pronounced in the MG than the TA muscle. (2) A net loss in total SDH activity/fiber was observed solely in FT fibres of the MG.
Musacchia et al. 1992	[180]	rat	tail harness	14d	Vastus Medialis: fiber and capillary density (caps/mm²), LDH and CS activities.	Fibre density in both homogeneous and mixed portions were sig. < SF portion; Capillary density in mixed portion was sig. < corresponding spaceflight mixed portion.	(1) There is an absence of type II fiber change in the VM following HS.
Ohira et al. 1992	[15]	rat	tail harness	14d	Soleus fibers: F-CSA, SDH and GPD activities.	Decline in Total SDH activity (SDH act.X CSA) in FT fibres; +Total GPD activity in FT fibres;	(1) ATPase activity was unaffected by HS. (2) The ATPase activity of hybrid fibres is comparable to that of type I fibres reflecting a dissociation between myosin isoform and ATPase activity within these hybrid fibres. (3) Individual soleus fibres emerge with a greater glycolytic p[rofile after 14d of HS.

Table IV: Muscle metabolic properties following periods of non-weight bearing activity in animals

Study	Ref #	Species	Model	Period	Method of Measurement	Results	Comments
Gallego et al. 1979	[186]	adult cat	casting & deafferented and/or cord transection (C-T)	14d	**Soleus:** AHP duration	Sig. dec. in **AHP duration** after S & C-T but the latter was **prevented** by L and not more affected by S. Mean duration of AHP was sig **correlated** with Sol muscle:BW ratio.	(1) In absence of sensory signals, certain motoneuron properties depend on the conditions of the innervated muscle (retrograde influence). (2) It appears that the primary factor responsible for the trophic influence is the metabolic change in muscle rather than contractile activity itself.
Fournier et al. 1983	[33]	rat	pinned joints @ different lengths	4w	**Sol and MG: IEMG:** Raw EMG signals were recorded simultaneously for 15 min/hr for 24 hrs. Recordings were 3 and 9 days after implantation and 7, 17 and 28 days after IM.	**Sol** iEMG after 4w IM was reduced similarly whether in N or L. Atrophic response betw. N & S was similar, but S caused ~80% decrement in iEMG. MG iEMg only affected in S ~50% decrement, but atrophy identical in both N & S.	(1) The observed alterations in the level of neuromuscular activity (iEMG) do not appear to be accountable for the atrophic changes and modified functional properties that ensue following immobilization @ different lengths.
Alford et al. 1987	[34]	rat	tail harness	3-28d	**Sol, MG and TA: MW** (absolute); **IEMG:** Raw EMG recorded simultaneously for 25 min/hr for 24 hrs. Recordings were 7 and 3d prior to HS and on days 1, 3, 7, 14, 21 and 28d during HS.	MW: Sol -49%, MG -28% and TA -3%; **EMG activity:** Prior to HS, **Sol** EMG 10X > MG and 20X > TA; **HS Day 1:** Sol -91%, MG -54%, TA +133%; **Day 7:** Sol -19%, MG -2%, TA +273%. Sol affected > MG.	(1) Daily amounts of EMG activity of Sol and MG decreased substantially on the day of suspension but recovered significantly to within presuspension values by day 7, and remained so throughout 28d suspension period. (2) TA activity remained elevated throughout the HS period and is likely due to the HS procedure. (3) Despite continuous atrophy, the Sol and MG regained their activity levels by the first week of HS.
Riley et al. 1990	[13]	rat	tail harness	4, 7, 10-14d	**Soleus:** EMG activity.	EMG activity shifted from tonic to phasic.	(1) The change in EMG activity pattern observed in the soleus may be a consequence of the altered use during hindlimb suspension.

Study	Ref #	Species	Model	Period	Method of Measurement	Results	Comments
Blewett and Elder 1993	[35]	rat	hindlimb	28d	**Sol and PL**: Chronic EMG recordings were quantitated over 24hr period for a total of 40d (control: 6d, HS: 28d, Recovery: 6d).	**Throughout** HS, activity levels remained well below control levels and by d28 were reduced almost equally in Sol (61%) and PL (65%). During recovery there was a return to control levels in Sol and a significant increase in PL but not to the control levels.	(1) Soleus and plantaris muscles displayed marked reductions in activity levels whereby normal periodic fluctuations in activity patterns were altered. Shifts between elevated and low levels of activity were replaced by significant increases in low-activity periods at the expense of the high activity periods.
Canu and Falempin 1997	[187]	rat	tail harness	7d	**Soleus** and **TA**: Intramuscular EMG recordings and analysis during locomotion @ increasing speeds.	HS led to a decrease in TA **burst duration** after 7d which may explain the latency increase between TA offset and Sol onset. **Afferent feedback** of Sol muscle from tendon (Ib) seems to be of great importance to TA burst duration.	(1) Locomotor patterns in the HS rats are affected by the reduction in afferent feedback from the Sol eus which consequently may result in hyperexcitable soleus motoneurons and a decline in TA activity by way of reciprocal inhibition.

Table V: Neural adaptations during periods of non-weight bearing activity in animals

was initially depressed by 63 and 50% in the Sol and 63 and 23% in the MG respectively during the first week of immobilization [33]. At the end of 4 weeks, however, the immobilized Sol muscle was unable to restore its normal activity pattern and maintained a 50% reduction from control values when fixed at a neutral length [33]. While immobilized in the shortened position, further deterioration in EMG activity, averaging 77% was seen [33]. In contrast, the MG demonstrated a full recovery to normal activity levels when fixed at neutral length while in a shortened position succeeded to increase only to 50% normal activity [33].

In agreement with the previous study, Alford et al. [34] reported significant alterations in the activity patterns of the rat Sol, MG and TA during HS. The EMG activity recorded in the Sol and MG was reported to decrease significantly by 91% and 54% respectively on the first day of suspension [34]. However, by day 7, both muscles had returned to within 81 and 98% of their pre-suspension activity levels despite continuous atrophy [34]. Therefore, the observed alterations in the level of neuromuscular activity (EMG) do not appear to be accountable for the atrophic changes and modified functional properties that ensue following immobilization at different lengths and HS. Unlike the Sol and MG, the TA muscle activity, a FT flexor, demonstrated a significant increase within 3 days following the onset of HS which remained elevated throughout the study. This response is most likely caused by either the absence of inhibitory inputs via reciprocal inhibition or the slight mechanical stretch imposed on the TA by the suspension procedure [34].

In contradiction to the above, Blewett and Elder reported the same trends following the onset of hindlimb suspension, however, the Sol and PL activity levels remained considerably below pre-suspension values [35]. By day 28, the Sol and PL activity patterns were practically equivalent to one another, demonstrating a 61 and 65% reduction, respectively [35].

Furthermore, the activity pattern of the Sol muscle was reported to shift from tonic to phasic type activity [13].

Part II: Structural and Functional Adaptations of Skeletal Muscle to Spaceflight in Animal Models

During spaceflight (SF), animal models are commonly used to study the effects of weightlessness on the musculoskeletal system in order to obtain a better understanding of the factors responsible for muscle atrophy. Data from a variety of studies using rat muscles have shown a great deal of variability attributed to actual flight and postflight conditions, as well as differences in methodology.

Morphological Observations

A review of the recent literature regarding the effects of spaceflight on the morphological properties of skeletal muscle in animal models are presented in Table VI. Comparable with HS, morphological changes appear swiftly following the onset of exposure to spaceflight but not uniformly among the different muscles. Again, extensors are more susceptible than flexors, and extensors consisting primarily of ST fibres, are more susceptible than those with a higher proportion of FT fibres. For example, relative Sol muscle mass (mg/100g body wt.) reportedly dropped 38% in both adult and juvenile rats, following a 5.4 day SF [9, 36], whereas the MG muscle only lost 16% of its relative mass and relative TA muscle mass was unaffected [36]. In addition, SF induces alterations in the size of both fibre types where type I fibres atrophy to a greater extent than type II fibres in ST muscles. Mean type I FCSA decreased by 24% in the Sol [37, 38] and 46% in the AL muscle [37],

Study	Ref #	Species	Flight	Period	Method of Measurement	Results	Comments
Martin et al. 1988	[39]	rat	Spacelab-3	7d	Sol, AL, PL, EDL, and MG: MW, FCSA; mATPase Histochemical Analysis.	MW: -Sol; FCSA: AL ST -46%, FT -36% > Sol; PL & EDL < Sol & AL; ATPase rxn: +% FT in AL >>Sol>MGd;	(1) The magnitude of the structural and metabolic responses that arise following SF are conditional upon the muscle, its fibrer-type composition and the initial size of those fibres.
Desplanches et al. 1990	[37]	rat	Cosmos-1667	7d	Sol and EDL: MW; mATPase Histochemical Analysis; CS, HAD, and LDH.	MW: Sol -23%, EDL -11%; Fibre %: Sol: - type I % counterbalanced by + in type IIa. FCSA: type I -24% no change in Caps or enzyme activities; EDL: -type I%, HAD -27%.	(1) Muscle atrophy of the soleus muscle following short-term SF is accompanied by a decrease in the percentage of type I fibres counteracted by an increase in the percentage of type IIa fibres.
Miu et al. 1990	[45]	rat	Cosmos-1887	12.5d	Sol and MG: FCSA; Myosin phenotype expression based on mATPase Histochemical Analysis.	FCSA: Sol atrophied 2x > MG, fibres in MGd (+type I%) > fibres in MGs (-type I%); Fast myosin expression Sol: 40% in SF vs. 22% in C, 31% hybrid in SF vs. 8% in C.	(1) The magnitude of enzymatic and cell volume changes that occur in response to SF, depend on numerous factors, including the muscle, the region within the muscle, and its fiber-type composition.
Riley et al. 1990	[41]	rat	Cosmos-1887	12.5d	AL, Soleus, PL and EDL: mATPase Histochemical Analysis and Ultrastructural analysis.	AL -36% and Sol -38% mean F-CSA; AL: 18%+ in hybrid fibers and a concomitant -ST fibres; Regional interstitial edema, segmental necrosis present in AL & Sol; +Ubiquitin staining in AL;	(1) The atrophic responses in the soleus and adductor longus muscle swere further exemplified by the pathological aberations within the fibres themselves. (2) The is evidence of fiber conversion with the observed increase in hybrid fibre percentage at the expense of ST fibres.
Musacchia et al. 1990	[46]	rat	Spacelab-3	7d	Soleus and EDL: MW (absolute) and F-CSA	Soleus: -20% absolute mass; -ST and FT CSA; +FT fiber density; EDL: -15% absolute mass; -FT CSA; +FT fiber density.	(1) Spaceflight effects on the muscle mass and FCSA are comparable in the soleus and EDL muscles after 1w but the effects are more pronounced in the soleus.
Bodine-Fowler et al. 1992	[40]	rhesus monkey	Cosmos-2044	2w	Sol, MG, and TA Biopsies: FCSA	Mean FCSA: SF had no effect in Sol and MG; slightly smaller in TA. Fiber types: Sol and MG no change, TA had +% of type II fibers.	(1) The absence of atrophic responses in the soleus and MG fibres of the rhesus monkey were noted. (2) The differential responses to SF between the rat and monkey may be associated with the species, the manner in which they were restrained.

Study	Ref #	Species	Flight	Period	Method of Measurement	Results	Comments
Jiang et al. 1992	[30]	rat	Cosmos 2044	2w	Single fibres of MG (FT ext) and TA (FT flex): FCSA, Fibers catagorized on basis of immunohistochemical rxn.	MG: +% hybrid fibers; TA: no hybrid fibers; +FT-CSA. Both: No effect on % fiber type composition. Mean fiber size of any type was unaffected in either muscle	(1) Evidence of fibre type conversion in the MG. (2) Mean fiber size of any type was unaffected and enzyme activities remained normal in either muscle.
Musacchia et al. 1992	[180]	rat	Cosmos 2044	2w	Vastus Medialis: fiber density (fibres/mm2), FCSA.	Mixed portion: Type I & II CSA reduced; Homogeneous portion: Reductions in type II also. Fiber density was increased.	(1) Exposure to SF induced significant reductions in the FCSA and increases in the fiber density of both regions of the VM of the rat.
Ohira et al. 1992	[15]	rat	Cosmos 2044	2w	Soleus fibers: F-CSA	Hybrid fibers+15% @ the expense of ST fibers; Mass -25%; Mean ST CSA -30%.	(1) Compared with HS, SF induced a greater percentage of hybrid fibres and a lesser effect on mean FCSA reduction in the soleus muscle.
Riley et al. 1992	[14]	rat	Cosmos 2044	2w	Adductor Longus (ST anti-G muscle): MW, mitochondrial content, F-CSA, Ultrastructural analysis. Fatty-acid binding protein (FABP) content.	Mean wet weight was normal~ due to interstitial edema; ST CSA decrease > FT; relative content of fast myosin LC was > slow myosin LC; fatty-acid binding depressed; elevated ubiquitin staining; ECC-like lesions 3x > HS; segmental necrosis; hybrid fibers.	(1) In response to SF, the presence of hybrid fibres displayed evidence of fiber type conversion which can be further exemplified by the observed drop in the FABP content. (2) The presence of hybrid fibers following HS, does not coincide with a drop in FABP content.
Henriksen et al. 1993	[9]	rat	STS-48	5.4d	Soleus and EDL: Relative mass, IFV assesed by inulin space.	Soleus: -38% in weight to BW after SF; IFV +52%. EDL: no statistical difference; IFV: unaltered.	(1) The observed increase in IFV following SF in the unloaded soleus is directly related to the decline in muscle weight and not reloading or reentry forces since the volume changes were present in the HS model without reloading.
Caiozzo et al. 1994	[38]	rat	STS-54	6d	Soleus: Biochemical: Immunohistochemistry of MHC isoforms and mATPase Histochemistry fiber typing.	MHC expression: de novo expression of +type IIx MHC w/ - type IIa; FCSA: -24% type I.	(1) Early onset of atrophic responses in the soleus were accompanied by the presence of fiber conversion (type IIa to IIx).

Study	Ref #	Species	Flight	Period	Method of Measurement	Results	Comments
Riley et al. 1996	[181]	rat	SLS-1 SLS-2	9d 14d	**Soleus, AL and EDL:** Myofiber areas, interstitial edema, Lesions. in-flight (IF), post-flight (PF).	**Myofiber areas:** SLS-2: decreased w/o recovery by 14d in both AL and Sol. **Interstitial edema:** None observed In-F but present in AL 2.3h post-F, Sol none Post-F. **ECC-like lesions:** detected in AL PF but absent IF.	(1) It appears that interstitial edema and sarcomere lesions are postflight occurences.
Talmadge et al. 1996	[4]	rat	Cosmos 2044	14d	**Soleus:** SDS page analysis and Immunohistochemistry of MHC isoforms.	Type IIx population @14d +4% ; Percentage of fibers with multiple MHCs increase 28%; @14d SF, 3%+ in type IIb (ns).	(1) Additional exposure to SF induces a further fiber type conversion from type IIx to IIb MHC.

Table VI: Muscle morphological observations following spaceflight in animals

whereas mean type II FCSA was reportedly unaffected in the Sol [38] and reduced by 36% in the AL muscle [37] following 7 days of SF.

In general, SF data, using both myofibrillar ATPase (mATPase) histochemistry and immunohistochemical procedures, show a tendency for gains in the type II [37, 39, 40] and/or hybrid [4, 15, 30, 41, 42] fibre populations in ST skeletal muscle following flights ranging from 1-2 weeks. Such gains in percentage usually coincide with a decrease in type I percentage, and like HS, SF appears to induce a shift in fibre types or conversion since the total number of fibres remain constant [15, 30, 37, 41]. Accordingly, after a 7 day flight, muscle atrophy of the Sol was accompanied by a decrease in the percentage of type I fibres, and counterbalanced by an increase in the percentage of type IIa fibres [37].

Categorized on the basis of mATPase histochemical and immunohistochemical reactions, similar reductions in the percentage of type I fibres were coupled with increases in hybrid fibre percentage in the AL [41] and Sol [42] muscles following a 12.5 day and 2 week SF, respectively. Furthermore, after a 12.5 day flight, both VI (ST extensor) and VL (FT extensor) muscles exhibited a change in the distribution of myosin isoform expression, according to mATPase histochemistry, with the preferential reduction in slow type I and intermediate (hybrid) forms of myosin being more prominent in muscles with a higher proportion of ST fibres [3]. That is, microgravity induces a shift in the myosin phenotype expression of the VI muscle from predominently slow to a higher proportion of fast myosin isoform [3].

Protein Composition

Zero gravity has also been reported to have significant effects on skeletal muscle protein composition. One week of SF exposure featured observations similar to HS, with reductions in total protein content (mg/muscle) in the Sol and a net accumulation of protein in the PL and MG muscles of young rats [36]. A review of the recent data on protein composition in rat hindlimb muscles during spaceflight are presented in Table VII.

Briefly, reductions in myofibrillar protein content have been observed primarily in the Sol [42] and VI [3, 43] muscles, whereas the VL, PL and TA muscles appear to be unaltered during SF [36, 42].

Regarding myosin (MHC) content, there appears to be a downregulation of slow type I and upregulation of fast type IIx or IIb MHC protein isoforms as a result of microgravity exposure. For example, following a 14 day SF, the Sol muscle responded with a 30% loss in pure type I MHC protein isoform and a dramatic increase (148%) in the amount of type IIx MHC protein isoform [42]. The VI muscle displayed qualitatively similar changes to the Sol in MHC isoform content, however, to a lesser degree [42]. These results are largely in support of the previous findings during simulated weightlessness, confirming the preferential degradation of slow type I myosin isoforms.

Therefore, weightlessness exerts a significant influence on the antigravity musculature. The early onset of atrophic responses are accompanied by changes in the contractile and speed-related properties which are mediated by the alterations in MHC content and phenotype expression, reductions in muscle mass, and preferential loss of contractile proteins.

Study	Ref #	Species	Flight	Period	Method of Measurement	Results	Comments
Baldwin et al. 1990	[3]	rat	Cosmos-1887	12.5d	VI and VI: ATPase activity, protein content, myosin distribution.	VI: -21% MW; -myofibril protein content; +myofibril ATPase activity; -slow myosin w/ +fast myosin (ns); -myosin content (mg/muscle) @ expense of S & Im isoforms. VI: MW, PC, ATPase activity were unaffected; Shift in myosin from -Im to + F type.	(1) There appears to be a change in the distribution of ATPase isomyosins with a reduction in the slow and intermediate forms of myosin being more pronounced in muscles with a higher proportion of ST fibres. (2) This reduction coincides with a decline in myofibril protein content.
Thomason et al. 1992	[84]	rat	Cosmos-2044	2w	VI, Triceps brachii (TB) and Lateral Gastrocnemius (LG): expression of a-actin mRNA and cytochrome c mRNA.	Skeletal a-actin mRNA: -25% in VI and -36% in LG; no change in TB. Cyt c mRNA: -36% in VI only.	(1) Exposure to prolonged SF can induce alterations in the pre-translational regulation of gene expression for myofibrillar proteins in skeletal muscle.
Riley et al. 1992	[14]	rat	Cosmos 2044	2w	Adductor Longus (ST anti-G muscle): MW, mitochondrial content, F-CSA, Ultrastructural analysis. Fatty-acid binding protein (FABP) content.	Mean wet weight was normal~ due to interstitial edema; ST CSA decrease > FT; relative content of fast myosin LC was > slow myosin LC; fatty-acid binding depressed; elevated ubiquitin staining; ECC-like lesions 3x > HS; segmental necrosis; hybrid fibers.	(1) In response to SF, the presence of hybrid fibres displayed evidence of fiber type conversion which can be further exemplified by the observed drop in the FABP content. (2) The presence of hybrid fibers following HS, does not coincide with a drop in FABP content.
Tischler et al. 1993	[36]	rat (juvenile)	STS-48	5.4d	Sol, PL, MG, and EDL: Relative MW, protein content and insulin sensitivity.	MW/100g BW: Sol -38%, MG -16%, TA and EDL ns; Protein Content: Sol -20%, PL and MG showed a net accumulation of protein; Insulin response on Glucose uptake: Sol +51%. Also, insulin increased uptake 2.5 -fold in Sol relative to 1.6 in controls.	(1) SF exposure featured reductions in muscle weight, protein content, a net accumulation of protein in the PL and MG and a boost in the sensitivity to insulin in the soleus muscle. (2) These responses were similar to those observed after HS of similar duration.
Haddad et al. 1993	[43]	rat	SLS-1	9d	VI and VI: MW, MHC protein content, MHC mRNA levels.	MW: -VI not VL; MHC: VI net loss: type I -40% and type IIa-IIx -19% with trace +type IIb; red VL net loss: type I -42% and type IIa-IIx -17% with type IIb +43%; MHC mRNA: VI type I -32%, type IIa -78%, type IIb +104%; red VL: type IIa -62%, type IIb +120%	(1) Following SF exposure, both VI and red VL exibited a downregulation of type I and IIa-IIx MHC isoforms with an upregulation of type IIb MHC isoform. (2) mRNA levels were consistent with this pattern with the exception of the type I MHC mRNA expression in the red VL which appeared to be unaffected.

Study	Ref #	Species	Flight	Period	Method of Measurement	Results	Comments
Esser and Hardeman 1995	[81]	rat	SLS-1	9d	Soleus and EDL: levels of slow and fast isoform mRNAs.	Slow isoform mRNAs: Soleus was variable, EDL no change; Fast mRNAs: All increased in both Sol and EDL.	(1) A general increase in fast isoform mRNA was demonstrated in both fast and slow muscles following exposure to SF.
Caiozzo et al. 1996	[42]	rat	STS-58/SLS-2	14d	Soleus: Contractile properties; Fiber type distribution catagorized on basis of immunohistochemical rxn; MHC protein/mRNA content: Sol, VI, PL and TA relative to SF + 14d recovery.	Sol: -25% myofibrillar protein content; -37% Po w/ +20% Vmax; F-Hz relation shifted right; -+IIx MHCmRNA; Sol > VI: -% type I w/ +% hybrids; VI:+type IIx MHC content w/ -type I MHC. VI MHC mRNAs: -type 1 & IIa, +type IIx & IIb. PL & TA: unaffected.	(1) The observed modifications in contractile and speed-related properties are mediated by the alterations in MHC phenotype expression and reductions in muscle mass. (2) Alterations in MHC mRNA content vs. MHCs at the protein level appear to be uncoupled which may be a consequence of the changes in protein turnover.

Table VII: Muscle protein composition following spaceflight in animals

Functional Properties

To date, very few studies have examined the effects of weightlessness on the *in situ* contractile properties of rat skeletal muscle; please refer to Table VIII. Alterations in P_o are most likely mediated by the preferential loss of contractile proteins [24]. Observations in the Sol muscle include, significant reductions in P_o by 24% and 37% and increases in Vmax by 14% and 20% following 6 [38] and 14 days [42] of SF, respectively. The observed increase in Vmax is consistent with the trend toward a greater proportion of fibres expressing fast MHCs.

Metabolic Properties

Data on the effects of microgravity on the rat Sol metabolic profile are variable. Metabolic studies on selected muscles of the rat following spaceflight are reviewed in Table IX. In summary, mean SDH activity has been reported to increase [44], decrease in type II fibres [15] or remain unchanged [39] following SF of 1-2 weeks duration. In contrast, mean GPD activity is commonly observed to increase in both fibre types [39] or exclusively in type II fibres [45]. In accordance with HS, capillary density is reported to increase [46] or remain unchanged [37]. Thus, controversy arises as to which direction the existing fatigue characteristics of a muscle will proceed following exposure to weightlessness.

Study	Ref #	Species	Flight	Period	Method of Measurement	Results	Comments
Rapcsak et al. 1990	[182]	rat: pregnant & male	Cosmos-1514 Cosmos-1667	5d & 7d	Forelimb and Hindlimb: MW, ATP-Ca$2+$-induced max tension, Speed of Contraction (Vc) and relaxation (Vr).	Weight Loss: +Sol, triceps and brachialis; Tension: -Sol and brachial; Vc & Vr: decreased in all muscles @5d w/ greater reduction @ 7d in Sol and brachialis.	(1) Short-term SF induced reductions in strength and increases in speed-related properties of forelimb and hindlimb muscles. (2) Differential responses were observed between slow and fast muscles.
Caiozzo et al. 1994	[38]	rat	STS-54	6d	Soleus: Contractile measurements: F-V and F-Hz relations and fatiguability; Biochemical: Immunohistochemistry of MHC isoforms and mATPase Histochemistry fiber typing.	F-V relationship: Po-24% and Vmax +14%; F-Hz relationship: shifted to right.; MHC : de novo expression of +type IIx MHC w/ - type IIa; FCSA: -24% type I; Fatiguability: -54% in force generated @ end of test.	(1) Early onset of atrophic responses in the soleus were accompanied by changes in the contractile and speed-related properties, the presence of fiber conversion (type IIa to IIx) and increased fatiguability.
Caiozzo et al. 1996	[42]	rat	STS-58/SLS-2	14d	Soleus: Contractile properties; Fiber type distribution catagorized on basis of immunohistochemical rxn; MHC protein/mRNA content: Sol, VI, PL and TA relative to SF + 14d recovery.	Sol: -25% myofibrillar protein content; -37% Po w/ +20% Vmax; F-Hz relation shifted right; -+IIx MHCmRNA; Sol > VI: -% type I w/ +% hybrids; VI:+type IIx MHC content w/ -type I MHC. VI MHC mRNAs: -type 1 & IIa, +type IIx & IIb. PL & TA: unaffected.	(1) The observed modifications in contractile and speed-related properties are mediated by the alterations in MHC phenotype expression and reductions in muscle mass. (2) Alterations in MHC mRNA content vs. MHCs at the protein level appear to be uncoupled which may be a consequence of the changes in protein turnover.

Table VIII: Muscle contractile properties following spaceflight in animals

Study	Ref #	Species	Flight	Period	Method of Measurement	Results	Comments
Martin et al. 1988	[39]	rat	Spacelab-3	7d	Sol, AL, PL, EDL, and MG: MW, FCSA; mATPase Histochemical Analysis; SDH, GPD, and ATPase activities.	MW: Sol most -; FCSA: AL ST -46%, FT -36% > Sol; PL & EDL < Sol & AL; ATPase rxn: +% FT in AL >>Sol>MGd; ATPase activity: + Sol; SDH: unaffected in ST & FT fibres of Sol, AL, PL, MG; EDL FT +SDH; GPD: + Sol & AL in ST & FT, EDL-ST+.	(1) The magnitude of the structural and metabolic responses that arise following SF are conditional upon the muscle, its fibre-type composition and the initial size of those fibres.
Miu et al. 1990	[45]	rat	Cosmos-1887	12.5d	Sol and MG: mATPase Histochemical Analysis, Total SDH and GPD activities (OD/min X CSA).	FCSA: Sol atrophied 2x > MG, fibres in MGd (+type I%) > fibres in MGs (-type I%); Total SDH activity: dec. in Sol; GPD activity: + in type II of Sol only; Fast myosin expression Sol: 40% in SF vs. 22% in C, 31% hybrid in SF vs. 8% in C.	(1) The magnitude of enzymatic and cell volume changes that occur in response to SF, depend on numerous factors, including the muscle, the region within the muscle, and its fiber-type composition.
Musacchia et al. 1990	[46]	rat	Spacelab-3	7d	Soleus and EDL: MW (absolue), Capillary density (caps/mm2) and F-CSA	Soleus: -20% absolute mass; -ST and FT CSA; +FT fiber density; + capillary density. EDL: -15% absolute mass; -FT CSA; +FT fiber density; + capillary density.	(1) Spaceflight effects on the muscle mass, FCSA and capillarity are comparable in the soleus and EDL muscles after 1w but the effects are more pronounced in the soleus.
Bell et al. 1992	[44]	rat	Cosmos-1887	12.5d	Soleus: Regional distribution of SDH.	SO: Alteration in entire distribution of SDH, both subsarcolemmal and intermyofibrillar regions. Increase in mean SDH activity after SF. FOG: SDH distribution in the subsarcolemmal region was reduced.	(1) Following 12.5 d of SF, both type I and IIa fibres of the soleus muscle exhibited alterations in the regional distribution of SDH, whereby, a reduction was observed in the subsarcolemmal region of these fibres.
Bodine-Fowler et al. 1992	[40]	rhesus monkey	Cosmos-2044	2w	Sol, MG, and TA Biopsies: FCSA and SDH activity.	Mean FCSA: SF had no effect in Sol and MG; slightly smaller in TA. Fiber types: Sol and MG no change, TA had +% of type II fibers; Mean SDH activity: Sol and TA no change, MG reduced postflight.	(1) The absence of atrophic responses in the soleus and MG fibres of the rhesus monkey were noted. (2) The differential responses to SF between the rat and monkey may be associated with the species, the manner in which they were restrained.

Study	Ref #	Species	Flight	Period	Method of Measurement	Results	Comments
Chi et al. 1992	[29]	rat	Cosmos 2044	2w	**Soleus and TA:** Metabolic Enzyme Assays: PFK, pyruvate kinase, GPD and Hexokinase being usually more abundant in type II (GLYCOLYTIC); MDH and KACAT are OXIDATIVE.	**Soleus fibers (same as HS):** Average fiber size (dry weight/length) -37%; pyruvate kinase, GPD and PFK (ns) were elevated; no change in KACAT; hexokinase was greatly increased. **TA fibers:** PFK, GPD were + in type IIa; MDH no change in any fiber type.	(1) Following a 2w SF, the soleus fibres emerge with an enzyme profile that has shifted slightly toward an elevated glycolytic capacity.
Jiang et al. 1992	[30]	rat	Cosmos 2044	2w	**Single fibres of MG (FT ext) and TA (FT flex):** FCSA, SDH, GPD, and ATPase activites; Fibers catagorized on basis of immunohistochemical rxn.	**MG:** +% hybrid fibers; + ATPase activity of FT fibers; +ATPase/SDH ratio in FT fibers. **TA:** no hybrid fibers; +FT-CSA.	(1) Evidence of fibre type conversion in the MG. (2) Mean fiber size of any type was unaffected and enzyme activities remained normal in either muscle.
Musacchia et al. 1992	[180]	rat	Cosmos 2044	2w	**Vastus Medialis:** fiber and capillary density (caps/mm**2**), FCSA, RNA concentration, LDH and CS activities.	**Mixed portion:** Type I & II CSA reduced; **Homogeneous portion:** Reductions in type II also. **Capillary & Fiber densities** were increased w/ capillary density being greater in mixed portion (// to portion of type I). **LDH** and **CS activities** showed no difference.	(1) Exposure to SF induced significant reductions in the FCSA and increases in the fiber and capillary densities of both regions of the VM of the rat.
Ohira et al. 1992	[15]	rat	Cosmos 2044	2w	**Soleus fibers:** SDH and GPD activities	Decline in Total SDH activity (SDH act. X CSA) in ST fibers; GPD activity was unaffected.	(1) Comparable with HS, SF reduced the oxidative capacity of individual ST fibres of the soleus muscle.
Ishihara et al. 1996	[183]	rat	STS-58/SLS-2	14d	**L5 Ventral Horn Neurons:** SDH activity and soma CSA.	No change in **mean CSA or mean SDH activity** or in the size distribution of neurons PF and recovery. Decrease in SDH activity in moderate-sized neurons, which persisted for 9d recovery.	(1) The SDH activity of moderately sized neurons in this specific region of the spinal cord appear to be affected by SF.
Yoshioka et al. 1997	[184]	rat	STS-58/SLS-2	14d	**MG, LG, PL, TA and EDL: Ulrastructural Analysis:** mitochondrial volume **Enzymatic analysis:** SDH and PFK activities of fast-type muscles.	**Mito volume:** no change in MG, LG, PL, TA or EDL; Structural alterations produced during recovery; **SDH activity:** + MG only and **PFK activity:** -PL only.	(1) The absence of change in the mitochondrial volume suggests that energy stores for muscle contraction may be preserved in fast skeletal muscle during SF.

Table IX: Muscle metabolic properties following spaceflight in animals

Part III: **Structural and Functional Adaptations of Human Skeletal Muscle to Non-Weight-Bearing Activity and Weightlessness**

In humans, several ground-based models have been utilized to mimic the effects of weightlessness. Bedrest (BR) eliminates body weightbearing of both lower extremities. Unilateral lower limb suspension (ULLS), where all ambulatory function is accomplished on crutches with an elevated sole on the shoe of one foot, has recently been employed to unload one lower limb.

Limb immobilization is limited as a model of unloading compared to ULLS and BR, due to the restrictions imposed on limb mobility by preventing lengthening and shortening contractions around the joint. Immobilization-induced atrophy in ambulatory humans by limb casting after trauma or surgery appears to be more profound than responses induced by ULLS or BR.

Morphological Observations - Non-Weight-Bearing Activity

Structural observations regarding the effects of immobilization on lower extremity human skeletal muscle include: marked atrophy of the antigravity musculature (quadriceps femoris and calf muscles) with progressive reductions in muscle and fibre CSA, and no significant changes in percentage fibre type distribution based on individual muscle biopsies. These observations are similar to the findings reported in the rat, with the exception of percentage fibre type distribution, where increases in the percentage of fibres expressing type II phenotypes are observed in the rat antigravity musculature. A selection of studies examining the effects of non-weight bearing activity and spaceflight in human skeletal muscle mass and structure are reviewed in Table X.

The quadriceps femoris muscle (QFM) of healthy individuals immobilized in a plaster leg cast for 4 weeks exhibited profound reductions

Study	Ref #	Human	Model	Period	Method of Measurement	Results	Comments
Sargeant et al. 1977	[48]	Male n=7 (healthy)	leg cast	*6-7w	QFM: Anthropometric Leg volume; FCSA; VO2max, VL. biopsy	Total leg volume: -12%; VO2 max: -17%; No change in % fibre type; FCSA: -46% type I, -37% type II; VO2 max is related to leg volume but FCSA reduction is proportionally > reduction in VO2.	(1) Due to the inactivity of the QFM following long term immobilization, extensive atrophy of type II and I fibres was evident and proportionately greater than the reduction in VO2.
MacDougall et al. 1980 [193]		Male n=7 (healthy)	elbow cast	5-6w	Triceps brachii: FCSA, % fiber distribution, PT @ 30°/s;	No change in % fibre type; FCSA: Training +39% FT and +31% ST, Immobilization -33% FT and -25% ST. PT: +98% post-training, -41% post-immobilization.	(1) Immobilization induced reductions in FCSA of both fibre types (type II > I), with a marked decrement in strength.
Lindboe and Platou 1984	[194]	Male n=10	leg cast @ 15°	3d	VM biopsies: FCSA	FCSA: type I -14.4% (NS) and type II -17.3% (NS).	(1) Non significant reductions in both fiber types of the VM were observed following short term immobilization.
Veldhuizen et al. 1993	[47]	Male n=6 Female n=2 (healthy)	leg cast	4w	QFM: MCSA by CT scan; VL biopsy;	CT scan: -21% of QFM CSA; Biopsy: -16% fibre diameter, resulting in -29% in mean FCSA, change in fibre distribution was not significant.	(1) Profound atrophic responses were present in the QFM by the marked reductions in MCSA and FCSA following 4w of non-weight-bearing immobilization.
Hather et al. 1992	[51]	Male n=5 Female n=3 (healthy)	ULLS	6w	QFM: MCSA by MRI and Biopsy of VL for FCSA and capillary density.	Total muscle CSA -12% whereas total thigh CSA did not change: KE CSA -16%, KF CSA -7%; RF not affected; Biopsies: fibre type % same; type I FCSA -12% & type II FCSA -15% (type IIa only); Capillary density +15%; # caps/unit area type I & IIa +15% and 14%.	(1) The decline in QFM CSA was accompanied by atrophy of both type I and IIa, where the magnitude of the response was likely contigent upon fibre size rather than type. (2) The increase in capillary density is likely due to the reductions in fibre CSA.
Berg et al. 1993	[62]	Male n=6 (healthy)	ULLS	4w	QFM: Biopsy of VL: Fiber composition and FCSA.	Non-significant change in fibre distribution and FCSA	(1) 4w of ULLS failed to induce significant alterations in fiber distribution and size according to biopsies of the VL muscle.

Study	Ref #	Human	Model	Period	Method of Measurement	Results	Comments
Adams et al. 1994	[64]	Male n=10 (healthy)	ULLS	16d	QFM: T1-weighted MR images for MCSA; VL biopsies for FCSA, fibre composition.	MCSA: -8% but Ham not affected; FCSA: tended to decrease (-9%) but non-significant; Fibre Composition: not altered by ULLS.	(1) After 16 days of ULLS, decrements in QFM CSA were observed based on MRI, however, biopsies of the VL exibited unsignificant results in terms of FCSA.
LeBlanc et al. 1988	[75]	Male n=9	Horizontal	5w	AE and AF: MRI and Isokinetic strength @ 60 deg/s.	CSA of AE (PF) -12%; AF CSA ns; Strength: AE -26%, AF ns.	(1) Differential muscle atrophy and a disproportionate reductions in strength relative to CSA were observed in response to 5w of BR.
Convertino et al. 1989	[53]	Male n=8	HDT	30d	QFM and Calf: MCSA by CT scan.	CSA: QFM -8.1% Fat +9.2%; Calf -4.8%, Fat -4%	(1) Computed tomography scans of the thigh and calf muscles demonstrate proportional decreases and increases in CSA and Fat, respectively.
Hikida et al. 1989	[55]	Male n=8	HDT	30d	VL and Sol biopsies: Histochemcal, Biochemical and Ultrastructural analysis.	STfibre %: VL-13.6%, Sol +8%; FCSA of both ST & FT were reduced in VL and Sol; Biochem: CS & HAD declined but more so in Sol than VL; Ultrastructure: Disorganized myofibrils, necrotic fibres, edema, regenerating satellite cells; Capillary density: Sol.	(1) Despite reductions in FCSA, capillary density decreased in the soleus muscle following BR and this correlated with a decrease in oxidative capacity. (2) Both soleus and VL muscles shared similar reductions in FCSA.
LeBlanc et al. 1992	[67]	Male n=8	Horizontal	17w	AE, AF, KE, KF: MRI: regional volumes; DPA: muscle mass; Nitrogen balance; 3-MeH.	Total lean body tissue loss: calculated from N balance, -3.9 kg; Loss occured mostly in lower limbs; MRI volumes: AE -30%, AF -21%, KE and KF -16-18%. Lower > Back muscles.	(1) Nitrogen balance, MRI and DPA measurements conclude that atrophic changes persist throughout the 119 d BR period with the lower extremities being primarily affected.
Berry et al. 1993	[54]	Male n=6	HDT	30d	QFM and Calf: MCSA by MRI scan.	Quadriceps lost uniformly 11% of its CSA and the triceps of the leg 10.5%, with 12.8% lost from the deeper soleus versus 8.5% from the gastrocnemius.	(1) Following prolonged BR, MRI scans of the QFM and Calf revealed similar reduction in CSA.
Ferrando et al. 1995	[52]	Male n=5	Horizontal (waterbed)	7d	Thigh and Calf: MRI muscle volume changes.	Thigh: -2.93% in muscle volume; Calf: -2.18% (p=0.07) in muscle volume	(1) MRI was able to detect significant reductions in the thigh muscle volume following 1w of BR.

Study	Ref #	Human	Model	Period	Method of Measurement	Results	Comments
Ferrando et al. 1996	[89]	Male n=6	HDT	14d	Body Comp.: DEXA: whole & lean body mass (LBM) and % body fat; Metabolic: plasma cortisol, testosterone, IGF-I and insulin concentrations, Urinary Nitrogen; Protein Metabolism: whole body and skeletal muscle protein synthesis(PS) and breakdown (PB).	Body Comp: Total BM same (-LBM, +fat mass); 73% loss in thighs and calves; Metabolic: no changes (no stress) except -N-balance (urinary N > 2nd wk BR); Protein Metabolism: skeletal -50% PS, PB ns change; whole body -13% PS w/ PB no change.	(1) Inactivity-induced loss of whole-body protein is primarily due to the decrement in skeletal muscle protein synthesis. (2) This protein synthesis decrease is not associated with metabolic stress or hormonal changes. (3) Absence of an observable change in protein breakdown in human muscle following 14d of BR reflects the difference between species and related protein metabolism.
Larsson et al. 1996	[57]	Male n=3	HDT	6w	VL Single Muscle fibres: Specific tension (Po/CSA) and Vo; myosin isoform expression and myofibrillar protein/MHC content (mg/muscle).	MHC composition: relative fibre types and MHC proportions were not affected, SDS-page showed 38% weaker staining for MHC bands, i.e. -MHC content; FCSA -12.7%; Specific Tension: type I -44.6%, IIA -41.5%, IIAB -31.8%. Vo from all fibres not affected.	(1) BR induced significant alterations in the contractile properties of single VL fibres with the drop in specific tension related primarily to the loss of myofibrillar proteins. (2) 6w BR had no effect on MHC composition and speed-related properties of the single VL muscle fibres.
Berg et al. 1997	[56]	Male n=7	HDT	6w	QFM: MCSA by MRI, VL Biopsy: FCSA, MHC composition;	Mean FCSA -17.6%, type I -18.2% only. No change in % type & MHC comp. Type IIB % > IIB MHC isoforms. QFM CSA -13.8%.	(1) Atrophic responses following 6w of BR do not include changes in fiber type composition and MHC expression.
Zhou et al. 1995	[61]	Male n=3 n=5	STS-32, -33 and 34	5d 11d	VL biopsies: Myosin Phenotype Expression: mATPase and immunohistochemistry and SDS-Page analysis.	Immunohisto: @ 11d: +% type II MHC, -% type I MHC; MHC composition of single fibers: 10% less fibers expressed only type I MHC; 42% + in fibers expressing only type IIa MHC (ns); 3% expressed all 3 MHCs. No significant alterations post 5d SF.	(1) Rapid changes in myosin phenotype expression of individual fibres, suggests fiber conversion and indicates adaptability of MHCs to periods of unloading with faster rates of change occurring with SF.

Study	Ref #	Human	Model	Period	Method of Measurement	Results	Comments
Edgerton et al. 1995	[60]	Male n=3 n=5	STS-32, -33 and 34	5d 11d	VL biopsies: mATPase Histochemistry, FCSA.	**Composition:** -6-8% type I and +9% in type IIa, no change type IIb. **FCSA:** mean -16-36% w/ type IIb>type IIa>type I.	(1) Alterations in structural properties of muscle fibres can take place rapidly with SF.
LeBlanc et al. 1995	[59]	Male n=2 Female n=2	STS-47	8d	**Calf, Thigh, and Lower back:** MRI for regional muscle volume changes	**24 h after landing:** Sol-gastroc (-6.3%), ant. calf (-3.9%), hamstrings (-8.3%), quadriceps (-6.0%) and intrinsic back (-10.3%) compared to baseline. At 2 weeks post **Recovery** the hamstrings and intrinsic lower back muscles were still below baseline.	(1) Decrements in lower extremity and back musculature size occur rapidly during exposure to uG.

Table X: Muscle morphological observations following periods of non-weight bearing activity and spaceflight in humans

in overall CSA by 21%, as measured by computerized tomography (CT) scan [47]. Biopsies from the VL muscles demonstrated similar reductions, with a 29% decrease in mean FCSA [47]. Following 6-7 weeks of immobilization, the VL muscle demonstrated extensive atrophy of type I and II fibres with reductions of 46% and 37%, respectively [48].

ULLS induces atrophic responses which are comparatively similar to, but proportionately less than immobilization. For example, magnetic resonance imaging (MRI) measurements of the QFM revealed a 6.8% and 16% decrease in whole muscle CSA at 4 and 6 weeks respectively [49-51]. In contrast, VL biopsies exhibited no alterations in FCSA at 4 weeks, however, mean type I and II FCSA decreased by 12 and 15% respectively after 6 weeks of ULLS [51]. Again, no alterations in percentage fibre type distribution were seen [51].

Horizontal or head-down tilt (HDT) BR have been commonly employed to induce neuromuscular adaptations to unweighting comparable with SF. Unlike ULLS, bedrest eliminates the weightbearing effect of both lower extremities, with HDT used to simulate the cephalid fluid shift response experienced during weightlessness.

Early onset of atrophic responses are observed after 1 week of horizontal BR where MRI was able to detect significant reductions in thigh muscle volume [52]. At 4 weeks of BR with HDT, an approximate 8-11% decrease was detected in the QFM CSA while the calf muscle CSA exhibited losses from 5-11% [53, 54]. Likewise, both VL and Sol muscle biopsies displayed significant losses in mean FCSA [55]. After 6 weeks of BR with HDT, a 13.8% reduction was noted in QFM CSA while both type I and II fibres of the VL muscle reportedly demonstrated 18% decreases in FCSA [56].

Once more, atrophic responses following BR did not include significant changes in VL muscle fibre type composition and/or MHC expression [56, 57], with the exception of one study where the type I percentage reportedly decreased by 13.6% in the VL muscle and increased by 8% in the Sol muscle following 4w of BR with HDT [55]. Biopsy specimens, however, are generally too small to detect any changes in myosin phenotype expression and the possibility of myosin isoform transition in a given fibre cannot be excluded [57].

Morphological Observations - Spaceflight

To date, there are limited data in the English language scientific literature on the effects of microgravity on the neuromuscular system. Alterations of the musculoskeletal system have been reported over the years beginning with the earliest Gemini, Apollo and Skylab missions. Postflight urinary increases in nitrogen, potassium, calcium and phosphorous, as well as reductions in anthropometric leg volume and weight loss have all been reported and considered indirect measures of skeletal muscle atrophy [58]. The following changes in the morphological properties of human skeletal muscle exposed to SF resemble the responses previously outlined in the rat.

Skylab missions ranged in duration from 28 to 84 days. Postflight analysis of Skylab astronauts showed a 7-11% reduction in leg volume, however, this loss was attributed to only 50% muscle mass and 50% cephalic fluid shift [58]. Recently, Leblanc et al. reported significant reductions in regional volumes of the calf and thigh using MRI 24 hours after landing following an 8 day SF [59]. Decrements in lower extremity size are further exemplified at the muscle fibre level, where biopsies obtained from 5 astronauts demonstrated significant reductions in mean FCSA of type I by

16%, type IIa by 23% and type IIb fibres by 36%, following an 11 day SF [60].

Observations also included alterations in percentage fibre type composition and myosin phenotype expression of the VL muscle following 11 days of SF [60]. Myosin ATPase histochemical analysis revealed a 6-8% decrease on average in the type I fibre percentage, with a 9% increase in type IIa percentage and no modifications in type IIb percentage [60].

In keeping with this trend, immunohistochemical analyses demonstrated a 10% reduction in fibres expressing pure type I MHC isoform while the percentage of fibres expressing type IIa MHC isoform rose 42% after exposure to microgravity [61]. In addition, 3% of the fibres expressed all 3 MHC protein isoforms suggesting the presence of hybrid fibres [61].

Together, these rapid changes in mATPase activity and myosin phenotype expression of individual fibres suggests fibre conversion and indicate the adaptability of myosin protein isoform to periods of weightlessness [60, 61].

Functional Properties - Non-Weight-Bearing Activity

Recent data regarding the effects of non-weightbearing activity and spaceflight on the functional properties of human skeletal muscles of the lower extremity are reviewed in Table XI. The findings are similar to the responses delineated in the rat with the exception of the speed-related properties which appear to decrease in the affected muscles of humans. Briefly, the profound atrophic responses in QFM mass and structure are accompanied by marked reductions in isokinetic strength (-52%) and endurance (-27%) parameters following 4 weeks of non-weightbearing immobilization [47]. During ULLS, isokinetic peak torque (PT) [49, 62, 63]

Study	Ref #	Human	Model	Period	Method of Measurement	Results	Comments
MacDougall et al. 1980	[193]	Male n=7 (healthy)	elbow cast	5-6w	**Triceps brachii:** PT @ 30°/s; FCSA	No change in % fibre type; FCSA: *Training* +39% FT and +31% ST, *Immobilization* -33% FT and -25% ST. **PT:** +98% post-training, -41% post-immobilization.	(1) Immobilization induced reductions in FCSA of both fibre types (type II > I), with a marked decrement in strength.
Duchateau and Hainaut 1990	[77]	Male Female (healthy)	thumb cast @ 45°	6-8w	**Adductor pollicis and First dorsal interosseous:** Motor unit: contractile properties, order of recruitment, firing rate @ recruitment and maximal firing rate.	**Contractile properties:** mu twitch force for AP & FDI -41% & -37%; CT: +16% and +13%; recruitment threshold (%MVC): +133% and +123%; max firing rate: -42% and -39%.	(1) A neural modification to disuse was exhibited by the marked reduction in the maximal firing rate of all the motor units (slow > fast) following immobilization. (2) More high-threshold motor units were recorded in disused muscles at a relative submaximal force signifying the loss in tension generation capabilities.
Veldhuizen et al. 1993	[47]	Male n=6 Female n=2 (healthy)	leg cast	4w	QFM: PT @ 60-300°/s. **Endurance capacity:** using one-leg cycling @different workloads (Max Power) and isokinetic protocol 200 KEx @ 20%MVC @ 180°/s (Max Work).	**KE strength (PT):** -52% @ 60o/s and decreases @ all other velocities; **Isokinetic endurance:** -27%; **Max aerobic power:** NS	(1) Profound atrophic responses were present in the QFM by the marked reductions in strength and endurance following 4w of non-weight-bearing immobilization.
Berg et al. 1991	[49]	Male n=6 (healthy)	ULLS	4w	**QFM Limb performance:** PT, angle specific torque (AST), radiological density (RD) and CSA by CT scan.	QFM CSA: -6.8% w/ ULLS; **RD:** decreased in both limbs; **CON & ECC PT and AST:** -22% and -16% still persisted @ 4d recovery w/ -11% and -7%.	(1) Reduction in CSA following 4w of ULLS resulted from the decrease in contractile protein content. (2) The decline in strength was proportionally greater than the reduction in CSA.
Dudley et al. 1992	[50]	Male n=5 Female n=3 (healthy)	ULLS	6w	**QFM and AE:** Isokinetic and isometric strength.	Mean KE-AST: -21% and @ 4d recovery; -15%	(1) Isokinetic strength and CSA of the QFM were reduced following ULLS, the loss being greater in strength than CSA.

Study	Ref #	Human	Model	Period	Method of Measurement	Results	Comments
Adams et al. 1994	[64]	Male n=10 (healthy)	ULLS	16d	QFM: Isokinetic Ecc and Con AST @ selected velocities; T1-weighted MR images for MCSA;	AST: -12% but no change in torque velocity relation; MCSA: -8% but Ham not affected;	(1) After 16 days of ULLS, only decrements in isokinetic strength and QFM CSA were observed.
Berg et al. 1993	[62]	Male n=6 (healthy)	ULLS	4w	QFM: Initial PT (IPT) for 5 CON contractions @ 180deg/s, Average PT (APT) and WORK calculated from 30 contractions; Biopsy of VL: capillarity; CS and PFK activities.	Mean APT for 3 bouts @ Post 1(1d), 2 (4d) & Rec (7w) was -17, 13, and 7% (Work capacity remains - after recovery). Fatiguability + @ Post 2; Non-significant change in capillaries/fibre type. CS activity: decreased; PFK activity: no change	(1) 4w of ULLS impairs the work performance and endurance capacity of the QFM, accompanied by a reduction in CS activity.
Ploutz-Snyder et al. 1995	[74]	Male n=4 Female n=3 (healthy)	ULLS	5w	QFM: strength (1 RM) and CSA and muscle involment during exercise using shifts in signal intensity of T2-weighted MRimages for quantification.	CSA: -14% w/ no change in Rt leg; Strength: 20%; % change in Strength>% change in CSA; MRI contrast shift: after ULLS Left QFM >> rt QFM.	(1) The atrophied QFM exhibited an increase in MRI contrast shift (due to the proportionally greater muscle mass involvement) when generating a relative amount of work after 5w of ULLS, suggesting additional muscle fiber recruitment and altered motor control, hence neural drive.
Berg and Tesch 1996	[63]	Male n=10 (healthy)	ULLS	10d	QFM: PT and rectified EMG during MVIC; EMG @ fixed (100N) level and max w-velocity (AVmax).	PT: -13.4% and recovered @ 4d; Max EMGq remaind unchanged but Submax EMGq +25% but recovered @4d; Torque/EMGq @ MVIC and SubMVIC -11% and -16%; AVmax: -8.7%	(1) After 10 days of ULLS, strength losses of the QFM were accompanied by a decrease in electromechanical efficiency, whereby, an increased neural drive was required to maintain a given submaximal force level.
Convertino et al. 1985	[195]	Male n=7	HDT	10d	Exercise Responses: VO2max, heart rate (HR), anaerobic threshold (AT).	VO2max -8.7% after BR; Submax and max HR +; anaerobic threshold decreased to 41% VO2max	(1) BR induced a significant reduction in exercise tolerance, exhibited by the dramatic decrease in anaerobic threshold in just 10 days.

Study	Ref #	Human	Model	Period	Method of Measurement	Results	Comments
Grigoryeva and Kozlovskaya 1987	[71]	Male n=14	HDT	120d	TA and sural triceps (ST): Velocity and strength properties: isokinetic @ 180, 120, 60 and 0 deg/s	Progressive decline in strength of ST. & TA muscles over range of velocities: ST. -40%. TA -28%. SF effects < BR effects due to countermeasure programs. Recovery time was 1.5-2 months.	(1) The atrophic responses in the Calf muscles were more severe following 120 days of BR than SF of similar duration.
Gogia et al. 1988	[65]	Male n=15	Horizontal	5w	Lower extremity muscles: Isokinetic torque @ 60 deg/s	Significant torque losses in all muscles except elbow EX (EE); Sol -24%; Gastroc. -26%; TA -8%; KF -8%; KE -19%; and EF -7%.	(1) Percentage torque losses were proportionally higher in the antigravity muscles, namely the Sol, Gastroc. and KE.
LeBlanc et al. 1988	[75]	Male n=9	Horizontal	5w	AE and AF: MRI and Isokinetic strength @ 60 deg/s.	CSA of AE (PF) -12%; AF CSA ns; Strength: AE -26%, AF ns.	(1) Differential muscle atrophy and a disproportionate reductions in strength relative to CSA were observed in response to 5w of BR.
Dudley et al. 1989	[66]	Male n=7	HDT	30d	KE and KF: in vivo Torque-Velocity relationship.	KE force output was decreased by 19%, KF - 6% (ns); When compare with #513, the relative change in FCSA (8-14%) were < than relative change in strength (19%)....altered neural input.	(1) BR induced decrements in strength are more critical in the KE than the KF and this response does not modify the in vivo torque-velocity relationship.
LeBlanc et al. 1992	[67]	Male n=8	Horizontal	17w	AE, AF, KE, KF: Isokinetic strength testing;	Isokinetic: Significant decrease in KE @ 1w, also thigh and calf during BR.	(1) Isokinetic strength reductions conclude that atrophic changes persist throughout the 119 d BR period with the lower extremities being primarily affected.
Suzuki et al. 1994	[76]	Male n=3/9 Female n=5/5	Horizontal	10d 20d	KE & KF: Muscle mass, CSA, MVIC (selected lower and upper limb muscles), VO2max, Urinary N2 and Creatinine secretion.	10d BR: These variables tended to decrease except for MM of rt arm which increased. No correlation between -KE strength and CSA of thigh. No correlation betweem sum total isometric strength of the leg and total MM. 20d BR: Similar trend as above but at a statistically significant level. No correlation between muscle strength and mass or strength and CSA.	(1) Decrements in isometric strength were observed in both antigravity and non-antigravity muscles following short and long-term BR. (2) The rate of strength changes did not correlate to the corresponding alterations in muscle mass or CSA. (3) The greater loss of strength may reflect an impairment of neuromuscular function.

Study	Ref #	Human	Model	Period	Method of Measurement	Results	Comments
Larsson et al. 1996	[57]	Male n=3	HDT	6w	**VL Single Muscle fibres:** Specific tension (Po/CSA) and Vo; myosin isoform expression and myofibrillar protein/MHC content (mg/muscle).	**MHC composition:** relative fibre types and MHC proportions were not affected; **SDS-page** showed 38% weaker staining for MHC bands, i.e. -MHC content; **FCSA** -12.7%; **Specific Tension:** type I -44.6%, IIA -41.5%, IIAB -31.8%. Vo from all fibres not affected.	(1) BR induced significant alterations in the contractile properties of single VL fibres with the drop in specific tension related primarily to the loss of myofibrillar proteins. (2) 6w BR had no effect on MHC composition and speed-related properties of the single VL muscle fibres.
Portero et al. 1996	[68]	Male n=12	HDT	4w	**TA and GM:** MVIC; Endurance testing; MPF shift (rate of decrease during fatiguing contraction)	**Endurance time:** GM -33.1%, TA -43.3%; **MVIC:** TA -15.3%, GM -20.5%; **MPF:** TA no change in MPF shift, GM MPF shift +64.6%.	(1) The GM muscle was greatly impaired following BR in terms of isometric strength in comparison to TA strength. (2) Fatiguability of the GM increased as exemplified by the dramatic increase in the rate of MPF decrease
Berg et al. 1997	[56]	Male n=7	HDT	6w	**QFM: Muscle function:** KE PT w/ isometric and isokinetic CON MVC (30-180°/s), Max angular velocity (AVmax); maxEMG.	MVIC -24.5%; **PT** -28.9%; **Max EMG** -19.4%, **submax EMG** +44.4%; submax Nm/EMG - 23.2%, no change in max Nm/EMG; **AVmax -** 5.1%; **Specific Tension:** -12.5%	(1) Decline in electromechanical efficiency with the observed decrements in isokinetic and isometric strength and reduced neural input to muscle at maximal loads. (2) Submax EMG increase is a reflection of the reduced specific tension of the atrophied QFM which now has to produce a larger relative tension to achieve a preset torque level. (3) Atrophic responses following 6w of BR do not include changes in Force-Velocity relations.
Kozlovskaya et al. 1981	[70]	Male	FE-2 FE-3	140d 175d	**Thigh and Calf:** Electromechanical efficiency (**Myotest**) Isometric Force/iEMG; **Isokinetic Torque @** 60 & 180 deg/s; Sensory system & Proprioceptive reflexes.	**FE-3: Myotest** iEMG doubled during a submax static contraction; **Isokinetic (180 deg/s);** dec.in Gastroc; **Sensory:** +sensitivity of T-reflex (- threshold) ; **accompanied by - max amplitude of reflex responses.**	(1) Reduced electromechanical efficiency: greater expenditure in muscle activation for a fixed submaximal load. (2) Altered functioning of the afferent system leads to impaired sensory feedback signals for motor responses.

Study	Ref #	Human	Model	Period	Method of Measurement	Results	Comments
Grigoryeva and Kozlovskaya 1987	[71]	Male n=6	Salyut-7	110-237d	**TA and sural triceps (ST):** Velocity and strength properties: isokinetic @ 180, 120, 60 and 0 deg/s.	Substantial decline in strength of ST and TA muscles over range of velocities: ST -20 to 28%. SF effects < BR effects due to countermeasure programs. Recovery time was 1.5-2 months.	(1) Decline in calf muscle strength was less severe after long-term SF than hypokinesia (120d BR); attributable to the efficacy of the physical countermeasures program onboard the flight.
Koslovskaya et al. 1990	[72]	Male n=25	Salyut6-7 Mir	60-360d	**Gastrocnemius:** Electromechanical efficiency (Myotest) Isometric Force/iEMG; **Isokinectic Torque @** 60 & 180 deg/s; **Sensory system & Proprioceptive reflexes.**	Decline in strength of the gastroc. and an increase of EMG cost of contraction; Proprioceptive elements and spinal reflex mechanisms were altered in which threshold decreases were accompanied by reductions in the max amplitude of reflexes.	(1) The severity of the calf muscle deficits in torque-velocity performance immediately postflight exhibited a negative correlation with the duration of the flight; credited to the efficiency of the onboad countermeasures program.
Hayes 1992	[73]	Male n=19	Shuttle flights: short long	<6d 9-11d	**Extensor and flexor Muscles:** (elbow, shoulder, trunk, knee, and ankle) Isokinetic strength measurements: PT during concentric and eccentric contractions.	**Postflight:** Con and Ecc TE &TF; Con KF; Ecc SF; **Recovery:** Con TE and Ecc TF; Con KE. **Return to Baseline:** all except Con TE and Ecc AE.	(1) Varied deficits in strength parameters are demonstrated in selected muscles, with the severity of these decrements being associated with the antigravity musculature.

Table XI: Muscle contractile properties following periods of non-weight bearing activity and spaceflight in humans

and angle-specific torque (AST) [49, 64] in the QFM decline significantly over a range of velocities and muscle action modes.

Likewise, BR induces profound muscle weakness with percentage torque losses proportionally greater in the postural musculature, namely the calf [65-68] and QFM [56, 65-67]. For example, concentric PT at 60°/s decreased by 19% in the QFM, whereas the calf muscle featured a deficit of 26% after 5 weeks of horizontal BR [65]. Single fibre studies from the VL muscle revealed significant reductions of 32-45% in specific tension (P_o/CSA), related primarily to the loss of myofibrillar proteins following 6 weeks of BR with HDT [57].

Finally, whatever the model used, fatiguability of the affected muscle appears to increase [62, 68], Vmax appears to decrease [56, 63] and torque-velocity relation appears to be unaffected [64, 66].

Functional Properties - Spaceflight

Measurements of both elbow and knee flexor and extensor strength were taken preflight and postflight in all the Skylab astronauts, during all three missions, using an isokinetic dynamometer in a supine position at 45°/s [69]. Force deficits in the elbow extensor and flexor muscles were minimal in comparison to the lower extremity musculature. Decrements in average PT were 21% and 25% in the knee extensors and 10% and 19% in the knee flexors following 28 day (Skylab 2) and 59 day (Skylab 3) spaceflights, respectively [69]. Therefore, greater force deficits were demonstrated in the extensor muscles of the lower extremities [69]. In contrast, force deficits were markedly improved in Skylab 4 astronauts, an 84 day mission, attributed to the development and efficacy of the physical exercise program onboard the flight [69].

Russian spaceflight missions lasting between 110-360 days reported similar force deficits in the thigh and calf muscles, with the severity of deficits inversely proportional to the duration of SF [70-72]. Postflight isokinetic PT measurements of the calf musculature reportedly declined by 20-28% over a range of velocities following long term SF onboard Salyut-7 [71]. More recently, long (9-11 days) and short (<6 days) duration Shuttle flights have reported varied deficits in isokinetic PT of selected upper and lower extremity extensor and flexor muscles, with the severity of these decrements being associated with the antigravity musculature [73].

Metabolic Properties - Non-Weight-Bearing Activity

For a review of the metabolic data from human biopsy samples of the VL and Sol muscles following non-weightbearing activity and spaceflight, please refer to Table XII. At present, a limited amount of metabolic data from human muscle samples following exposure to periods of unloading exists. From this, however, we have surmised that, analogous to the rat, muscle fatiguability increases [62, 68] and is likely the result of a reduced oxidative capacity [55, 60, 62]. For example, following ULLS and BR with HDT, CS activity reportedly declined in the VL [55, 62] and Sol [55] muscles with the observed decrement being greater in the Sol than VL muscle.

Metabolic Properties - Spaceflight

Postflight histochemical analyses of biopsies taken from the VL muscles of astronauts, following 11 days of SF, revealed no change in SDH activity in either fibre type, while GPD activity appeared to increase by 80% in the type I fibres [60]. In addition, myofibrillar ATPase/SDH activities ratio, a sensitive indicator of fatiguability, is reportedly increased by 17% in type I

Study	Ref #	Human	Model	Period	Method of Measurement	Results	Comments
Wroblewski et al. 1987	[196]	Male n=6 Female n=6	leg cast @ 45°	6w	VL: X-ray microanalysis to record intracellular electrolyte concentrations.	[Cl]:+100% after surgery and 6w immobilization; [Na] also tended to increase (NS) over the same interval. No difference in [K], [P], or [S] after surgery and immobilization.	(1) Post-surgical rise in intracellular Cl concentration persisted throughout the immobilization period, possibly mediated by a reduction in the sarcolemmal Na/K ATPase pump activity induced by the decline in muscular activity.
Hather et al. 1992	[51]	Male n=5 Female n=3 (healthy)	ULLS	6w	QFM: Biopsy of VL for FCSA and capillary density.	Biopsies: type I FCSA -12% & type II FCSA -15% (type IIa only); Capillary density +15%; # caps/unit area type I & IIa +15% and 14%.	(1) The increase in capillary density is likely due to the reductions in fibre CSA.
Berg et al. 1993	[62]	Male n=6 (healthy)	ULLS	4w	QFM: Biopsy of VL: capillarity; CS and PFK activities; WORK calculated from 30 contractions.	Fatiguability + @ Post 2; Work capacity remains decreased after recovery; Non-significant change in capillaries/fibre type. CS activity: decreased; PFK activity: no change	(1) 4w of ULLS impairs the endurance capacity of the QFM, accompanied by a reduction in CS activity.
Hikida et al. 1989	[55]	Male n=8	HDT	30d	VL and Sol biopsies: Histochemical, Biochemical and Ultrastructural analysis.	Biochemical: CS & HAD activities declined but more so in Sol than VL; Capillary density: -Sol.	(1) Capillary density decreased in the soleus muscle following BR and this correlated with a decrease in oxidative capacity.
Edgerton et al. 1995	[60]	Male n=3 n=5	STS-32, 33 and 34	5d 11d	VL biopsies: mATPase Histochemistry, SDH and GPD activity, Capillarity (caps/fibre).	Enzymes: ATPase activity + type II; SDH no change; GPD +80% type I; Ratios: ATPase/SDH +17% type I &+38% type II; Caps/fiber: -24%. 5d SF non-significant	(1) Alterations in metabolic properties of muscle fibres can take place rapidly with SF. (2) Decrease in capillarity agrees with the increased fatiguability.

Table XII: Muscle metabolic properties following periods of non-weight bearing activity and spaceflight in humans

fibres and 38% in type II fibres [60], suggesting an increase in muscle fatiguability. Consistent with these data, a 24% decrease in capillarity (capillaries/fibre) was reported [60].

In general, these findings suggest that ULLS, BR and SF induce alterations in the normal metabolic profile of unloaded human skeletal muscle, whereby, reductions in oxidative capacity give rise to muscle with a greater reliability on anaerobic metabolism [60].

Neural Adaptations - Non-Weight-Bearing Activity

Alterations in neuromuscular activity induced by periods of unweighting and SF may also contribute to decrements in muscular force [50, 56, 63, 70, 72, 74]. Following prolonged periods of unweighting, human skeletal muscle groups of the lower extremities repeatedly exhibit decrements in force production which do not correlate with the corresponding changes in muscle mass [49, 50, 66, 74-76]. In general, the decline in strength of the knee [49, 50, 66, 74, 76] and ankle [75] extensors is proportionately greater than the reduction in muscle CSA. Clearly, factors aside from muscular atrophy appear to be responsible for the observed losses in force production. Investigators speculate that the greater loss in strength may reflect an impairment of neuromuscular function, hence, altered neural input or a decrease in neural drive [56, 66, 74-76]. Observations previously noted in the rat model either supported [35] or refuted [34] this suggestion. Recent studies investigating the neuromuscular adaptations that arise in human skeletal muscle following periods of unloading and spaceflight are reviewed in Table XIII.

Briefly, Duchateau et al. [77] reported a marked reduction in the maximum firing rate of both ST and FT motor units (ST>FT) following 6-8

Study	Ref #	Human	Model	Period	Method of Measurement	Results	Comments
Duchateau and Hainaut 1990	[77]	Male Female (healthy)	thumb cast @ 45°	6-8w	Adductor pollicis and First dorsal interosseous: Motor unit: contractile properties, order of recruitment, firing rate @ recruitment and maximal firing rate.	Contractile properties: mu twitch force for AP & FDI -41% & -37%; CT: +16% and +13%; recruitment threshold (%MVC): +133% and +123%; max firing rate: -42% and -39%.	(1) A neural modification to disuse was exhibited by the marked reduction in the maximal firing rate of all the motor units (slow > fast) following immobilization (2) More high-threshold motor units were recorded in disused muscles at a relative submaximal force signifying the loss in tension generation capabilities.
Grana et al. 1996	[78]	Male n=4 Female n=2 (healthy)	leg cast @ 35-45°	4w	Soleus: Neuromuscular Transmission w/ single fibre EMG (SFEMG) for latency variability of reponses (jitter) was calculated as mean of the consecutive differences (MCD).	Increase in MCD values after 4w of immobilization. they returned to normal level after 4w of recovery. That is, an increase in the rise time of EPP, this increment in the EPP rise time is measured as an increase in neuromuscumar jitter.	(1) Altered behaviour of the NMJ resulted following 4w of immobilization as demonstrated by an increase in the rise time of the EPP, commonly refered to as neuromuscular jitter.
Dudley et al. 1992	[50]	Male n=5 Female n=3 (healthy)	ULLS	6w	QFM and AE: Isokinetic and isometric strength, MRI , EMG.	Mean KE-AST: -21% and -15% @ 4d recovery; Mean KE CSA: -16% and 17%<control; Max iEMG of VL: -43% but MPF ns; Mean AE CSA: -18%; MPF: for GM and Sol was decreased.	(1) Isokinetic strength and CSA of the QFM were reduced following ULLS, the loss being greater in strength than CSA. (2) ULLS appears to decrease maximal iEMG of the VL and shift the MPF to lower frequencies.
Ploutz-Snyder et al. 1995	[74]	Male n=4 Female n=3 (healthy)	ULLS	5w	QFM: strength (1 RM) and CSA and muscle involment during exercise using shifts in signal intensity of T2-weighted MRimages for quantification.	CSA: -14% w/ no change in Rt leg; Strength: -20%; % change in Strength>% change in CSA; MRI contrast shift: after ULLS Left QFM >> rt QFM.	(1) The atrophied QFM exhibited an increase in MRI contrast shift (due to the proportionally greater muscle mass iinvolvement) when generating a relative amount of work after 5w of ULLS, suggesting additional muscle fiber recruitment and altered motor control, hence neural drive.

Study	Ref #	Human	Model	Period	Method of Measurement	Results	Comments
Berg and Tesch 1996	[63]	Male n=10 (healthy)	ULLS	10d	QFM: PT and rectified EMG during MVIC; EMG @ fixed (100N) level and max w-velocity (AVmax).	**PT: -13.4% and recovered @ 4d; Max EMGq remaind unchanged but Submax EMGq +25% but recovered @4d; Torque/EMGq @ MVIC and SubMVIC -11% and -16%; AVmax: -8.7%**	(1) After 10 days of ULLS, strength losses of the QFM were accompanied by a decrease in electromechanical efficiency, whereby, an increased neural drive was required to maintain a given submaximal force level.
Portero et al. 1996	[68]	Male n=12	HDT	4w	TA and GM: MPF shift (rate of decrease during fatiguing contraction)	**MPF: TA no change in MPF shift, GM MPF shift +64.6%.**	(1) Fatiguability of the GM increased as exemplified by the dramatic increase in the rate of MPF decrease or MPF shift.
Berg et al. 1997	[56]	Male n=7	HDT	6w	QFM: maxEMG activity; KE PT w/ isometric and isokinetic CON MVC (30-180°/s)	**Max EMG: -19.4%, submax EMG +44.4%; submax Torque/EMG ratio: -23.2%, no change in max Torque/EMG ratio; MVIC -24.5%; PT -28.9%; Specific Tension: -12.5%.**	(1) Decline in electromechanical efficiency with the observed decrements in isokinetic and isometric strength and reduced neural input to muscle at maximal loads. (2) Submax EMG increase is a reflection of the reduced specific tension of the atrophied QFM which now has to produce a larger relative tension to achieve a preset torque level.
Kozlovskaya et al. 1981	[70]	Male	FE-2 FE-3	140d 175d	Thigh and Calf: Electromechanical efficiency (Myotest) Isometric Force/iEMG; Isokinectic Torque @ 60 & 180 deg/s; Sensory system & Proprioceptive reflexes.	**FE-3: Myotest iEMG doubled during a submax static contraction; Isokinetic (180 deg/s): dec.in Gastroc; Sensory: +sensitivity of T-reflex (- threshold) ; accompanied by - max amplitude of reflex responses.**	(1) Reduced electromechanical efficiency: greater expenditure in muscle activation for a fixed submaximal load. (2) Altered functioning of the afferent system leads to impaired sensory feedback signals for motor responses.

Study	Ref #	Human	Model	Period	Method of Measurement	Results	Comments
Koslovskaya et al. 1990	[72]	Male n=25	Salyut6-7 Mir	60-360d	**Gastrocnemius:** Electromechanical efficiency **(Myotest)** Isometric Force/iEMG; **Isokinectic Torque @** 60 & 180 deg/s; Sensory system & Proprioceptive reflexes.	Decline in strength of the gastroc. and an increase of EMG cost of contraction; Proprioceptive elements and spinal reflex mechanisms were altered in which threshold decreases were accompanied by reductions in the max amplitude of reflexes.	(1) The severity of the calf muscle deficits in torque-velocity perfromance immediately postflight exhibited a negative correlation with the duration of the flight; credited to the efficiency of the onboad countermeasures program.

Table XIII: Neural adaptations during periods of non-weight bearing activity and spaceflight in humans

weeks of immobilization. This neural modification was further exemplified by the observed increase in the recruitment of high-threshold motor units at a relative submaximal force, also signifying the loss in tension generation capacity [77]. Altered behavior of the neuromuscular junction has also been observed following 4 weeks of immobilization, as demonstrated by an increase in neuromuscular jitter [78].

Six weeks of ULLS, reportedly decreases maximal integrated EMG of the VL muscle by 43% and shifts the mean power frequency (MPF) to lower frequencies [50]. However, after 10 days of ULLS, maximal EMG was reportedly unaffected while an increase in submaximal EMG activity was required to maintain a given submaximal force level [63]. This increase in submaximal EMG activity implies a decrease in electromechanical efficiency (ratio of force to EMG), where additional motor units are recruited, due to the reduced specific tension of atrophied fibres, for the maintenance of submaximal force production [56, 63, 74].

Likewise, BR with HDT also appears to induce reductions in neuromuscular efficiency, where a 19% reduction in maximal EMG was accompanied by a 44% increase in submaximal EMG activity of the QFM [56] and a MPF shift to lower frequencies was noted using EMG spectral analysis of the gastrocnemius muscle [68].

Neural Adaptations - Spaceflight

Neural adaptations following actual spaceflight indicate postflight increases in integrated EMG activity during the performance of a standard submaximal isometric contraction [70, 72, 79] and a shift of the power

spectrum to lower frequencies [80]. Once more, this signifies a reduced electromechanical efficiency where a greater expenditure in muscle activation is necessary for the exertion of fixed load [70, 72].

Part IV: Proposed Mechanisms of Muscle Atrophy in Response to Altered Loading States

In general, the precise physiological mechanism(s) responsible for muscular atrophy have yet to be determined. Information regarding the proposed mechanisms regulating the adaptability of skeletal muscle to periods of altered weight-bearing activity and weightlessness is largely obtained from ground-based animal research. A selection of studies investigating the possible mechanisms associated with non-weightbearing activity and spaceflight in rats and humans are reviewed in Table XIV.

Evidence suggests that mechanical activity exerts a powerful influence on the regulation of protein metabolism in skeletal muscle [3, 42, 81, 82]. That is, both protein synthesis and degradation processes are modified during conditions of unweighting which is consistent with the reduction in the protein composition of postural muscles.

Muscle proteins are repeatedly being remodeled via protein turnover and the quantity of protein maintained in the muscle is dependent on the net balance between protein synthesis and degradation. Evidence suggests that the rate of protein synthesis (a translational process) is significantly reduced following the onset of HS and is responsible for the early marked changes in myofibril protein levels in the Sol muscle [42, 82, 83]. This decline in protein synthesis rate (fraction/day) persists over the next few days until a new lower steady-state is achieved [83]. Two days prior to this plateau, however, the decline in protein synthesis rate is combined with a marked increase in the

Study	Ref #	Species	Model	Period	Method of Measurement	Results	Comments
Thomason et al. 1987	[2]	rat	tail harness	4d - 56d	Soleus: MW; Myofibrillar ATPase activity; myofibril protein content; MHC isoform expression.	Myofibril ATPase activity: no change; Protein content: -80%; Slow myosin (SM) content: -84%; Myosin isoform expression: -SM with equal +fast myosin (FM) isoform. Loss of myofibrillar protein occured to a greater extent than soleus mass loss.	(1) A significant shift in the myosin isoform profile from predominently slow myosin towards fast myosin expression. (2) This shift was mediated by the net degredation of SM isoforms and apparent de novo synthesis of the fast isoforms.
Thomason et al. 1989	[83]	rat	hindlimb unweighting	5h & 1w	Soleus: Protein synthesis rate : [^{3}H] Leu and estimate the time course of changes in myofibril synthesis and degredation rates. Measured B-myosin heavy-chain mRNA concentration to investigate the role of transcriptional regulation.	Sol exibits a dec. in total mixed & myofibril protein synthesis rates within 5 hr of HS (ns). By 7d, Sol muscle exhibits a significant decrease in both total mixed (46%) & myofibril protein synthesis rates (59%). No decrease in [B-myosin HC mRNA] @7d.	(1) A rapid but ns. reduction in the synthesis rate of myofibril protein is observed resulting in a net loss of myofibril protein in the soleus muscle. (2) This decrease in the synthesis rate persists over the next 7 days leading to significant losses in myofibril protein. (3) This decline in the rate of myofibrillar synthesis does not appear to be related to transcriptional regulation.
Taillandier et al. 1993	[17]	rat	tail harness	3 wk	Soleus: Ks; protein synthesis rate, total protein content, fiber-type distribution and F-CSA, T$_3$ circulation levels of Soleus. Rats fed a high-HP (30%) or medium-MP (15%) protein diet.	HS reduced Ks (33%) in MP but not HP. HP diet had no benefit in preventing atrophy; 65% dec. in FCSA. HP diet maintained higher % of type I in HS, whereas, HS-MP rats had 42% dec. in type I w/ inc. in type IIa & interm. HP induced dec. in T3 level.	(1) Both high and moderate-protein intake had no effect on the atrophic responses induced by HS. (2)During HS, the intake of a high-protein diet circumvented the reduction in Ks and partially conserved the fiber-type distribution in the Soleus. (3) A possible mechanism for the high-protein effects may include an hormonal response involving thyroid hormones.
Ku and Thomason 1994	[87]	rat	tail harness	18h	Soleus: Isolated polysomes by size fractionation on a liquid sucrose density gradient and hybridization.	Distribution of polysomes shifts to larger sizes indicating more ribosomes/mRNA during HS compared to Controls.	(1) The decrease in Soleus muscle protein synthesis rate is caused by the slowing of the nascent polypeptide chain elongation rate during HS.

Study	Ref #	Species	Model	Period	Method of Measurement	Results	Comments
Taillandier et al. 1996	[82]	rat	tail harness	9d	**Soleus:** MW, protein content (PC), rates of protein breakdown, proteolytic enzyme activities and mRNA profiles.	MW: -55%; **PC:** -53% (**contractile** -51% and **soluble** -50%); **total protein breakdown:** +66%, **Proteolytic pathways:** lysosomal & $Ca2+$-dependent proteolysis: +254%; **mRNA levels:** +cathepsin B, L & D, B-myosin mRNA: NC, +ubiquitin mRNA, + proteasome subunits mRNA.	(1) During exposure HS, both lysosomal and Ca^{2+}-activated proteinases make a minor contribution to the overall muscle proteolysis. (2) There is strong evidence, at the mRNA level, to suggest that an ATP-ubiquitin-dependent pathway is chiefly responsible for the increased protein degradation in the unloaded soleus.
Thomason et al. 1996	[88]	rat	tail harness	8h	**Soleus:** Polysome isolation by centrifugation and SDS-PAGE analysis for heat-shock protein 70 (hsc70)	**Soleus** : -29% in hsc70 levels associated with the polysomes and ATP levels +17% following 8h of HS. ATP levels > 1 mM affect the associative action of hsc70 with the polysomes.	(1) A decrease in heat-shock protein70 may decrease soleus muscle protein synthesis rate by decreasing the nascent polypeptide chain elongation rate. (2) Association of Hsc70 to the nascent polypeptide chain appears to be influenced by intracellular ATP levels.
Baldwin et al. 1990	[3]	rat	Cosmos-1887	12.5d	**VI and VI:** ATPase activity, protein content, myosin distribution.	VI: -21% MW; -myofibril protein content; +myofibril ATPase activity; -slow myosin w/ +fast myosin (ns); -myosin content (mg/muscle) @ expense of S & Im isoforms. VI: MW, PC, ATPase activity were unaffected; Shift in myosin from -Im to + F type.	(1) There appears to be a change in the distribution of ATPase isomyosins with a reduction in the slow and intermediate forms of myosin being more pronounced in muscles with a higher proportion of ST fibres. (2) This reduction coincides with a decline in myofibril protein content.
Thomason et al. 1992	[84]	rat	Cosmos-2044	2w	**VI, Triceps brachii (TB) and Lateral Gastrocnemius (LG):** expression of *a*-actin mRNA and cytochrome c mRNA.	**Skeletal-*a*-actin mRNA:** -25% in VI and -36% in LG; no change in TB. **Cyt c mRNA:** -36% in VI only.	(1) Exposure to prolonged SF can induce alterations in the pre-translational regulation of gene expression for myofibrillar proteins in skeletal muscle.

Study	Ref #	Species	Model	Period	Method of Measurement	Results	Comments
Haddad et al. 1993	[43]	rat	SLS-1	9d	VI and VL: MW, MHC protein content, MHC mRNA levels.	MW: VI not VL; MHC: VI net loss: type I -40% and type IIa-IIx -19% with trace +type IIb; red VL net loss: type I -42% and type IIa-IIx -17% with type IIb +43%; **MHC mRNA: VI** type I -32%, type IIa -78%, type IIb +104%; red VL: type IIa -62%, type IIb +120%	(1) Following SF exposure, both VI and red VL exibited a downregulation of type I and IIa-IIx MHC isoforms with an upregulation of type IIb MHC isoform. (2) mRNA levels were consistent with this pattern with the exception of the type I MHC mRNA expression in the red VL which appeared to be unaffected.
Stein et al. 1993	[188]	Male n=6	SLS-1	9.5d	**Protein Metabolism:** Nitrogen balance and whole body protein synthesis rate.	**Nitrogen Balance:** -67% in 4 crew; **Whole body protein synthesis rate:** +30% in 6 crew.	(1) Both the negative N-balance and protein synthesis increase indicate that a stress response occured and is likely part of the adjustment process to a uG environment. (2) The negative N-balance was likely due to the increase in protein breakdown rate which was greater than the increase in protein synthesis rate.
Esser and Hardeman 1995	[81]	rat	SLS-1	9d	**Soleus and EDL:** levels of slow and fast isoform mRNAs.	**Slow isoform mRNAs:** Soleus was variable, EDL no change; **Fast mRNAs:** All increased in both Sol and EDL.	(1) A general increase in fast isoform mRNA was demonstrated in both fast and slow muscles following exposure to SF.
Caiozzo et al. 1996	[42]	rat	STS-58/SLS-2	14d	**Soleus:** Contractile properties; Fiber type distribution catagorized on basis of immunohistochemical rxn; MHC protein/mRNA content: **Sol, VI, PL and TA** relative to SF + 14d recovery.	**Sol:** -25% myofibrillar protein content; -37% Po w/ +20% Vmax; F-Hz relation shifted right; -+IIx MHCmRNA; **Sol > VI:** -% type I w/ +% -+IIx MHCmRNA; **VI:**+type IIx MHC content w/ -type I hybrids; **VI:**+type IIx MHC content w/ -type I MHC. **VI MHC mRNAs:** -type 1 & IIa, +type IIx & IIb. **PL & TA:** unaffected.	(1) The observed modifications in contractile and speed-related properties are mediated by the alterations in MHC phenotype expression and reductions in muscle mass. (2) Alterations in MHC mRNA content vs. MHCs at the protein level appear to be uncoupled which may be a consequence of the changes in protein turnover.

Study	Ref #	Species	Model	Period	Method of Measurement	Results	Comments
Ferrando et al. 1996	[89]	Male n=6	HDT	14d	Body Comp.: DEXA: whole & lean body mass (LBM) and % body fat; Metabolic plasma cortisol, testosterone, IGF-I and insulin concentrations, Urinary Nitrogen; Protein Metabolism: whole body and skeletal muscle protein synthesis(PS) and breakdown (PB).	Body Comp: Total BM same (-LBM, +fat mass); 73% loss in thighs and calves; Metabolic: no changes (no stress) except -N-balance (urinary N > 2nd wk BR); Protein Metabolism: skeletal -50% PS, PB ns change; whole body -13% PS w/ PB no change.	(1) Inactivity-induced loss of whole-body protein is primarily due to the decrement in skeletal muscle protein synthesis. (2) This protein synthesis decrease is not associated with metabolic stress or hormonal changes. (3) Absence of an observable change in protein breakdown in human muscle following 14d of BR reflects the difference between species and related protein metabolism.
Stein et al. 1996	[189]	Male n=7-11	SLS-1 SLS-2	9.5d 15d	Nitrogen balance: Protein intake - urinary N excretion; Whole body protein synthesis: Ammonia and urea method; Fibrinogen synthesis.	N retention: -72%; during SF but the magnitude of the decrease lessened toward end of mission; Whole body protein synthesis: +35% @ FD 8; Fibrinogen synthesis: trend for an increase but ns until reentry.	(1) The onset of uG is characterized by a metabolic stress response accompanied by a rapid increase in protein turnover. (2) This marked increase in protein turnover arises early but returns to within a normal range by the 12th day.
Stein and Schluter 1997	[90]	Male n=9	SLS-1 SLS-2	9.5d 15d	Protein metabolism: Nitrogen Balance and urinary 3-methylhistidine (3-MH)- useful measure of myofibrillar protein breakdown.	N retention: -67%; 3-MH excretion: unchanged. Therefore no increase in muscle protein breakdown. In contrast to Skylab data but Agrees w/ BR data showing reduction in skeletal muscle mass occurs via a decrease in protein synthesis.	(1) 3-MH excretion was unresponsive to BR and SF, therefore, it is likely that myofibrillar protein breakdown does not appear to be a factor responsible for the observable reductions in muscle mass in humans.
Strollo et al. 1998	[190]	Male n=4	?	~5d ?	Hormones levels: salivary and urinary Testosterone (T); plasma ACTH, Cortisol, LH, T and its peripherally active metabolite 3ADG.	ACTH and CS levels: did not exceed the the referrence ranges accepted for normals @ any time during the study. T levels: salivary, urinary and plasma awa 3ADG were decreased during SF while LH levels were increased.	(1) Absence of hormonal responses normally identified with acute stress. (2) Hypoandrogenism arises in the absence of hypogonadotrophism and may explain the increase in LH secretion due to the lack of negative feedback from the reduced amount of circulating androgens.

Table XIV: Mechanistic studies in animals and humans following non-weight bearing activity and spaceflight

rate of protein degradation [83]. The subsequent increase in the protein degradation rate continues to rise and consequently is chiefly responsible for the major loss of myofibril protein in the non-weightbearing Sol muscle [83]. Exposure to prolonged SF can induce alterations in pre-translational regulation of gene expression for myofibrillar proteins as well as metabolic proteins [84]. Specifically, skeletal α-actin messenger RNA (mRNA) was reduced by 25% and 30% in the VL and lateral gastrocnemius (LG) muscles respectively, while cytochrome c mRNA declined by 36% in the VI muscle [84].

Factors responsible for the signal cascade initiating the decline in protein synthesis and subsequent rise in degradation rates, influencing the degree of atrophy associated with muscle unloading, remain unrecognized. However, protein expression in skeletal muscle appears to be somewhat linked to the activity of growth factors and hormones [85], which in turn, can influence the pre-translational, translational and post-translational regulation involved in gene expression [86]. For example, slow myosin isoform expression can be upregulated or downregulated depending on the circulating levels of thyroid hormones (T_3) [17]. These findings suggest that mechanical activity associated with weightbearing may influence the sensitivity of slow MHC gene to thyroid hormones [17].

In pursuit of the mechanism that initiates a rapid decline in protein synthesis in non-weightbearing muscle, Ku et al. [87] reported that a reduction in the nascent polypeptide elongation rate may be responsible in the early stages of declining synthesis rates in the unloaded Sol. In addition, a decrease in polysomal hsc 70, a regulatory component that guides the nascent chain through the ribosome channel by adhering to it, may account for this delay in the elongation rate [88]. It appears that polysomal hsc 70 is an ATPase and consequently, its regulatory role in protein synthesis is highly

sensitive to ATP levels [88]. With intracellular ATP levels on the rise in the Sol muscle during HS, Thomason et al. [88] suggest that protein synthesis machinery may sense altered functional demands via an ATP-sensitive mechanism involving hsc 70.

The rise in protein degradation rate (fraction/day) initially lags behind the rapid decline in synthesis rate in the Sol following the onset of HS [83]. This increase persists over a two week period, accounting for the net reduction in protein accumulation and myofibrillar protein content within the unloaded musculature [3, 82, 83]. The apparent uncoupling between reductions in myosin isoform expression at the protein level and myosin mRNA content being unaffected, are likely the consequence of protein degradation or post-translational events [42, 43].

Proteolytic enzyme activities are observed to increase in the Sol muscle during HS [82]. Once more, the exact mechanisms responsible for the initiation of theses proteolytic pathways and how they relate to non-weightbearing activity, remain to be elucidated. According to Taillandier et al. [82], both lysosomal and Ca^{2+} activated proteinases make minor contributions to the overall muscle proteolysis during exposure to HS. Based on strong biochemical evidence at the mRNA level, however, these investigators suggest that an ATP-ubiquitin dependent pathway is principally responsible for the increased protein degradation in the unloaded Sol muscle [82].

Thus far, limited data are available concerning the effects of unloading on human protein metabolism. Recent evidence suggests that loss of muscle mass is primarily due to the decrement in skeletal muscle protein synthesis [89]. Contrary to what has been acknowledged in the rat, protein breakdown does not appear to be a factor responsible for the observable reductions in muscle mass in humans [89, 90]. Indeed, more research is required to

provide additional insight into the mechanisms responsible for muscle atrophy following periods of mechanical unweighting and spaceflight.

Part V: Countermeasures

Microgravity-induced atrophy of lower limb musculature continues to be a significant problem for astronauts following prolonged spaceflight. In the future extended visits to orbiting space and planetary stations are being planned. It is anticipated that the average mission duration aboard the International Space Station will be 30-60 days. Therefore, more efficient approaches to counteracting the observed microgravity-induced pathophysiological changes must be explored in order to ensure astronaut health and safety during both long-duration spaceflight and upon reentry into Earth's gravitational field.

At present, countermeasures such as daily physical exercise regimens with the aid of various devices [91] and neuromuscular electrical stimulation (NMES), a transcutaneous application of electrical stimulation to selected muscles [92, 93], have been developed to attenuate microgravity-induced muscle atrophy. A wide variety of onboard exercise equipment has been developed for use in weightlessness, and when used appropriately appears to minimize physical deconditioning and microgravity-induced muscle atrophy [91]. During spaceflight missions, the 2-4 hour inflight regimen of daily exercise is time consuming and extremely costly to both life-support facilities and the operational workday of astronauts [91, 93]. Therefore, it is essential to design a more efficient and cost effective method of preventing microgravity-induced disuse muscle atrophy.

Exercise paradigms currently used in spaceflight are only moderately successful in sustaining proper physiological function and alleviating muscle atrophy [91]. Therefore, proposed ground-based investigations have been undertaken to study the efficacy of various new devices and exercise modalities [91] alone [22, 31, 32, 94-103] or in combination with anabolic/pharmacological agents [21, 104-106].

Recently published studies evaluating the effectiveness of various new approaches to counteracting the atrophic responses of the unloaded Sol and Gastrocnemius muscles of the rat during HS and SF are presented in Table XV. Results of these studies can be summarized as follows: mild periodic weight support activity (treadmill walking) during HS either prevented [96] or diminished the atrophic responses in the unloaded Sol [31], while responses the MG were only partially alleviated [32, 96]. Daily endurance type exercise (treadmill running) minimized muscle fibre atrophy and maintained oxidative potential in type II fibres in the Sol [95]. Resistive exercise training (grid climbing) was highly efficacious in maintaining Sol muscle mass and function while having a lesser effect on the MG [94]. Finally, eccentric exercise training protocols attenuated 80% and 44% of the relative Sol mass and protein content, respectively [22].

Data indicate that rats exposed to microgravity experience repeatable decrements in growth hormone (GH) plasma levels [107-110], brought about by microgravity-induced secretory dysfunction at the level of the pituitary somatotroph [107-110]. Having a direct impact on muscle protein synthesis, hormone replacement therapy, by exogenous treatment with anabolic agents such as clenbuterol, recombinant human GH (rhGH) and insulin-like growth factor I (IGF-I), has also been proposed as a countermeasure to non-weight bearing atrophy.

Clenbuterol-treated rats demonstrated less atrophy of the Sol [111], while rhGH administration had a slight prophylactic effect on Sol mass [112], or no effect at all [113]. However, combined treatment of rhGH with daily regimens of resistive exercise maintained myofibrillar protein content [21] and preserved muscle mass [104] to a greater extent than exercise alone. In addition, rhGH administration coupled with IGF-I and daily resistance muscle

Study	Ref #	Species	Model	Period	Method of Measurement	Method of measurement	Results	Comments
Gardiner and Lapointe 1982	[191]	rat	immobilization	28d	Electrical stimulation via sciatic nerve	**Sol, Gastoc and Plantaris:** MW/bw ratios; contractile properties.	MW/bw: ES had no attenuating affect on Sol, G and PL; **Contractile properties:** ES prevented certain speed related properties and increased the relative contractile tension @50Hz and endurance in the Gastoc.	(1) This stimulation protocol consisting of powerful muscular contractions was inadequate in preventing the atrophic responses induced by immobilization.
Savolainen 1987	[20]	rat	immobilization	1w & 3w	Electrical stimulation via sciatic nerve	**Gastroc, Sol and TA:** MW, Protein content, Proteolytic enzyme activities: Cathepsin D (CD), *B*-glucuronidase (*B*-g), alkaline protease (AP), acid autolysis (AA).	MW: @ 1w : less loss in stimulated G and TA, not @ 3w; **Protein Content:** @ 1w less dec. in TA; **Enzymes: CD** - only G @ 1w; *B*-g: + in TA and G @ 3w; **AP:** - in G @ 3w and + in TA @3w; **AA:** - in G and + TA (NS).	(1) Acid and alkaline responses induced by immobilization of the hindlimb musculature are differentially influenced by simultaneous treatment with electrical stimulation.
Appell 1986	[175]	mouse	immobilization	1, 4, 7, 11, 14, 18 d	Treadmill running for 7d pre and post Imm.	**TA:** Morphometric and Structural Analysis using EM: fibre CSA.	**Post-Training:** reduced the atrophy by 22%; **Pre-Training:** reduced atrophy by 30%.	(1) Post-training was less efficacious in ameliorating the atrophic responses to immobilization than the pre-training regimen.
Hauschka et al. 1988	[31]	rat	HS	7d	Periodic weight support: treadmill walking @ 0.83 m/s @ 19% incline for 10 min every 6 hr (total of 40 min/day)	**Soleus:** MW/bw, FCSA and Enzyme activities: SDH (OD/min X CSA).	MW/bw: HS -28%, HS-WS ns; **FCSA:** HS ST-36%, FT-34%, HS-WS ST -20% and FT -12% (sig > HS); **iSDH:** HS ST -28%, FT -ns, HS-WS ST -25% but FT ns.	(1) Intermittent weight support during HS ameliorated the atrophic responses in the unloaded soleus, with FT fibres being more sensitive to the training regimen than ST fibers.
Herbert et al. 1988	[94]	rat	HS	7d	Grid climbing (1m) inclined at 85o for 8 reps with a load =75% bw attached repeated 4X daily.	**Sol and MG:** MW/bw; Po and *in situ* contractile properties.	MW/bw: *Sol* HS < Con, HS-Ex = Con; *MG* both HS groups< Con; **Po:** *Sol* -44% in HS and -27% in HS-Ex and Ex significantly > HS, *MG* -23% in HS and -16% in HS-Ex. No exercise effect on fatiguability in Sol or MG	(1) Unloaded soleus muscle is more responsive to short-duration, resistive exercise training program with benefits to the structural and functionalproperties.
Graham et al. 1989	[32]	rat	HS	7d	Periodic weight support: treadmill walking @ 0.83 m/s @ 19% incline for 10 min every 6 hr (total of 40 min/day)	**MG:** FCSA and Enzyme activities: SDH, GPD (OD/min) & (OD/min X CSA).	MW/bw: HS -17% and HS-WS -13%; **FCSA:** HS: deep FT and ST -40%, HS-WS: Maintainance of ST and FT in both regions, Con>HS-WS>HS; **iSDH:** HS deep both types reduced but NS; HS-WS deep and superficial FT and deep ST were similar to CON;**iGPD:** no diff.	(1) Intermittent weight support during HS alleviated the atrophic responses induced by HS in the medial gastrocnemius muscle with the extent of responses related to the fiber type and region within the muscle.
Graham et al. 1989	[95]	rat	HS	28d	Treadmill for 1.5 hr/d @ 20 m/min and 30% grade.	**Soleus:** Fibre-type distribution, FCSA, integrated SDH activity (activity or (OD/min) x CSA)	MW (mg): -70% HS and -30% HS-Ex; **% FT fibres:** +19% HS and +17% in HS-Ex; **FCSA:** FT -46% HS and -18% HS-Ex, ST -69% HS and -48% in HS-Ex. **ISDH:** net loss in both fibres of HS but only a loss in ST of HS-Ex.	(1) Daily treadmill exercise partially attenuated muscle fiber atrophy and completely maintained the oxidative potential in FT fibres in the soleus during long-term HS.

Study	Ref #	Species	Model	Period	Method of Measurement	Method of measurement	Results	Comments
Pierotti et al. 1990	[96]	rat	HS	7d	Intermittent weight support (HS-WS): slow treadmill walking (0.2 m/s) @ 19% incline for 10 min every 6 hr.	Sol and MG: MW/bw; in situ contractile properties.	MW/bw: Sol -21% in HS and -9% in HS-WS (ns diff from Con); MG -19% in HS and -10% in HS-WS; Po: Sol HS-WS>HS w/ Po/MW in HS-WS = Con; MG: only HS showed a sig dec in Po, but Po/MW +14%.	(1) Low intensity, periodic treadmill exercise was capable of completely preserving soleus muscle weight and contractile function, while medial gastrocnemius weight and function were partially preserved.
Kirby et al. 1992	[22]	rat	HS	10d	Eccentric exercise via manual lengthening during ES.	Soleus: MW and Protein content and concentration (noncollagenous)	% Atrophy attenuated: 80% in relative MW and 74 % in absolute MW; 44% Protein content.	(1) Minimal amount of eccentric exercise training was required to partially preserve the protein content of the soleus muscle during HS.
Apostolakis et al. 1980	[192]	rat	immobilization	15d	Post-immobilization treatment with rhGH for 15d	Gastrocnemius: MW, EMG activity (iEMG), Contractile capacity and work performance.	MW: +47% for atrophied G and +19% for contralateral control leg; iEMG: +73% in atrophic G compared to untreated G; Tension: +58 (twitch) and +65% (tetanic) in atrophic G compared to untreated G; Work capacity: 62% of normal vs. 43% in untreated.	(1) Post-treatment with rhGH demonstrated significant gains in the recovery of the gastrocnemius muscle in terms of muscle weight, neuromuscular activity, contractile capacity and work performance.
Jiang et al. 1993	[113]	rat	Spaceflight	4d	rhGH administration	Sol: MW; fibre type composition; FCSA and enzyme activities.	MW: rhGH had no effect on the reduction; FCSA: rhGH had no effect in either group. Enzyme activities: SF or rhGH administration had no effect.	(1) Short-term exposure to SF induced atrophic responses that were uninfluenced by the administration of rhGH.
Apseloff et al. 1993	[111]	rat	HS	14d	aminohydroxybutane bisphosphonate (AHBuBP) for bone and Clenbuterol for muscle.	Sol and EDL: MW, protein concentration and enzyme activity.	Clenbuterol treated: ameliorated atrophy in Sol but had no effect on EDL, however, treated EDL was atrophic compared w/ saline HS rats; CS activity was significantly < unsuspended but > suspended untreated.	(1) Clenbuterol attenuated the loss of slow soleus muscle mass, enhanced exercise tolerance and diminished loss of CS activity, BUT, may have induced atrophy of EDL muscle.
Bigard et al. 1994	[112]	rat	HS	25d	Recombinant human GH (rhGH)	Sol: MW, fibre type distribution, FCSA, enzyme activities	Fibre type Dist: rhGH did not alter the HS-induced decrease in type 1%. FCSA: rhGH did not alter the HS-induced decrease in type I FCSA.	(1) During HS, treatment with rhGH had a slight prophylactic effect on the MW/bw of the Soleus muscle.
Grindeland et al. 1994	[104]	rat hypophysect	HS	7d	rhGH, R-Exercise (ladder climbing) and rhGH+Exercise	Extensors and Flexors: protein concentrations and FCSA.	GH treatment: maintained MW of the fast extensors (MG, LG, and Pl) and flexors (TA and EDL) but Sol and AL were unaffected: Exercise: no effect on any muscle; GH+Ex: +FCSA of fast and slow fibres of MG, +Sol MW and FCSA of slow fibres of Sol.	(1) Both rhGH therapy and exercise individually have a minimal effect on the maintenance of muscle mass during HS of hypophysectomized rats. (2) rhGH treatment in conjunction with exercise have positive interactive effects and preserved the mass and attenuated losses in FCSA.

Study	Ref #	Species	Model	Period	Method of Measurement	Method of measurement	Results	Comments
Linderman et al. 1994	[21]	rat	HS	5d	rhGH, Resistance Exercise (ladder climbing) and rhGH+ Resistance Exercise	Sol and Gastroc: MW, myofibrillar protein content and synthesis.	MW: Sol unaffected, EX attenuated Gastro and PI w/ and w/out GH; Protein content: Ex/GH attenuated loss of Myofibrillar content in G by 70%; Protein Synthesis: Ex/GH increased myofibrillar and mixed synthesis by 47 and 36% in gastrocnemius.	(1) Combined treatment of rhGH and daily regimens of resistive exercise maintained the myofibrillar protein content of the gastrocnemius muscle during short-term exposure to HS.
Linderman et al. 1995	[105]	rat	HS	3d	Resistance Exercise and rhGH+ Resistance Exercise	Sol and Gastroc: MW, myofibrillar protein synthesis rate (Ks) @ 15, 60, 180 and 360 min post-Ex.	MW/BW: HS no affect; Ks: Sol (>60%) > G (<40%); Post Soleus Ks: Ex +150% vs HS-NoEx @ 60 min post EX. and ns from Controls, GH-Ex + Ks 15, 60, and 360' post Ex vs no-Ex; Post G Ks: R-Ex did not significantly + Ks in either group. (unlike previous study)	(1) No evidence of rhGH effects on the resistive-exercise induced stimulation of protein synthesis, 1 hr post-exercise in the soleus. (2) Protein synthesis in the gastrocnemius was unaffected by exercise or rhGH treatment up to 6 hr post-exercise, suggesting a delayed response is more likely.
Allen et al. 1997	[179]	rat	HS	14d	rhGH/IGF-I, Resistive Ex (grid climbing) and combo of both.	Soleus: analysis of myonuclei by TDT labelling: indicator of apoptosis.	Check suspension.	(1) Combined treatment with GH-/IGF-I + resistance exercise attenuated the HS-induced loss of myonuclei and significantly decrease the number of fibres with morphologically abnormal nuclei.
Allen et al. 1997	[106]	rat	HS	14d	rhGH/IGF-I, Resistive Ex (grid climbing) and combo of both.	Sol: MW; Myonuclear number; FCSA; MHC profiles.	MW: GH/IGF-1 no effect, Ex partially, GH/IGF-1+Ex restored to control level; FCSA: HS-55%, Ex-44%, GH/IGF-1-49%, GH/IGF-1+Ex-23%; Myonuclear #: GH/IGF-1+Ex not different from Control.	(1) Daily resistance muscle loading and GH/IGF-1 administration have synergistic effects that attenuate the atrophic responses of unloaded muscle.

Table XV: Countermeasures to microgravity-induced muscle atrophy during periods of non-weight bearing activity in animals

loading completely preserved Sol muscle mass and greatly attenuated muscle fibre atrophy during 14 days of HS [106].

In humans, the use of high intensity resistance type activity, including eccentric contractions, has been given considerable attention [97, 99-103]. A list of recently published ground-based investigations of exercise countermeasures in humans during simulated microgravity are presented in Table XVI. Briefly, a variety of isokinetic and/or isotonic heavy resistance protocols have preserved overall muscle thickness [99] and fibre size [103], maintained or increased average work capacity [100] and preserved maximal strength performance [101, 102] of the knee extensors during BR with HDT. Although preliminary, these results will benefit the development of future countermeasures which will likely incorporate both endurance and dynamic resistance training protocols for the maintenance of normal physiological function during spaceflight.

Another proposed countermeasure to eliminate or minimize disuse atrophy is the use of NMES of selected muscles using an apparatus called "TONUS" developed by the Russian space program [114]. NMES is the transcutaneous application of an electrical stimulus to selected muscles that elicits a muscular contraction as a result of the percutaneous stimulation of peripheral nerves. The application of electrical stimulation with surface electrodes creates an electrical field, where the depth of penetration is proportional to the intensity of the stimulus [115]. Depending on the type of protocol, stimulus parameters, and pattern of stimulation (e.g. chronic vs. intermittent), NMES induces specific changes in muscular properties [116, 117].

The recruitment order of motor units initiated by direct motor nerve stimulation is described as follows: the largest motor units which innervate FT fibres and have larger axons, are recruited at the lowest current intensities

Study	Ref #	Human	Model	Period	Method of Measurement	Method of measurement	Results	Comments
Tesch et al. 1990	[98]	Male n=18	none	19w	Heavy resistance isotonic training w/ Con and Ecc/Con actions	Leg Press: 3RM with Con contractions and Con/Ecc contraction	**Post-training 3RM leg press:** Con 3RM leg press: Con group +12% and Con/Ecc group +22% (Con/Ecc grp > Con grp); Con/Ecc 3RM leg press: Con grp ns and Con/Ecc grp +26%. **Net total O2 uptake:** No difference between Con and Con/Ecc grps.	(1) Con/Ecc heavy resistance training represents a more effective and efficient muscle training regimen by producing greater force development during exercise and greater strength gain after training with minimal added energy cost.
Ellis et al. 1993	[99]	Male n=19	HDT-BR	30d	Isotonic (ITE) and Isokinetic (IKE) Exercise training: two 30min sessions/day for 5d/wk during BR	**Muscle Thickness:** Anterior thigh (RF and VI) and posterior leg (combined Sol = FHL+ TP) by ultrasonography	**Sol+FHL+TP group:** ITE nor IKE training influence rate or magnitude of decrease in thickness; **RF:** ITE and IKE maintained thickness during BR; **VI:** only ITE training maintained thickness while IKE showed a significant decline. All muscles in no-Ex grp dec.	(1) Posterior antigravity muscle group were not affected by either exercise regimen. (2) Both exercise regimens preserved RF unlike VI whereby only the ITE Ex maintained its thickness.
Greenleaf et al. 1994	[100]	Male n=19	HDT-BR	30d	Isotonic (ITE) and Isokinetic (IKE) Exercise training: two 30min sessions/day for 5d/wk during BR	KE and KF: Average total work; Peak torque.	**After BR: KE** average total work -16% in NOEx, +27% in IKE and unchanged in ITE; **KF:** No significant changes in either group. **Average PT:** no changes in KE or KF; **Muscular endurance:** unchanged during BR with all 3 regimens.	(1) Knee extensors were more responsive to the isokinetic exercise regimen during BR with a combined increase of 43% in work performance over the no exercise group.
Germain et al. 1995	[101]	Male n=12	HDT-BR	28d	Daily maximal intensity isokinetic + isometric exercise training (supine) w/ LBNP for 20d.	**QFM strength:** PT @ range of velocities including O deg/s	**Post BR:** A statistically significant decrease in mean PT occured @ most velocities in the untrained group, whereas, the values remained constant in the trained group.	(1) LBNP in conjunction with maximal isokinetic and isometric exercise training can be a highly efficacious in maintaining the force-velocity properties of the QFM during BR.
Ferrando et al. 1997	[102]	Male n=11	HDT-BR	14d	Isotonic resistance training of the KE every other day (6 days total) during BR (supine).	**QFM:** 1 RM strength and Muscle protein Synthesis (MPS) using phenylalanine tracer on muscle biopsies taken from the VL.	**MPS:** -46% in BR only and maintained in BREx grp. **1RM:** Preserved in BREx grp.	(1) Moderate isotonic resistance training can counteract the reduction in protein synthesis and preserve muscle strength in the VL muscle during BR.
Bamman et al. 1998	[197]	Male n=16	HDT-BR	14d	Near Maximal Isotonic resistance training every other day (6 days total) during BR. 5 sets of ~8reps of Con/Ecc Leg press @ 80-85% 1RM	**QFM:** 1-RM strength by leg press, MVIC and rmsEMG activity of VL, VM, RF, semi-T and BF; **VL biopsy:** MHC composition by immunohistochemistry, FCSA	**FCSA:** NoEx: type I -14.6%, type II -17.4% and mean -16.8%; Ex: All preserved. **MHC comp:** nc in MHC I, IIa, or IIx within groups but *SDS-page* -7% in type IIx % in Ex; **T-RM:** NoEx -9%, Ex nc; **MVIC:** NoEx -15%, Ex -13%; **EMG ratio (EMG/PT):** Ex -19%.	(1) High-intensity, isotonic concentric and eccentric resistive exercise of the QFM during BR completely preserved the VL muscle fibre size but the improvements in strength deficits were specific to the mode of training.

Table XVI: Countermeasures to microgravity-induced muscle atrophy in humans

while the smallest motor units, innervating ST fibres which have smaller axons, are only recruited at the higher current intensities [118]. That is, submaximal electrical stimulation preferentially activates the larger axons which have a lower input resistance in comparison to smaller axons. With supramaximal stimulation, all axons are activated and all motor units contract [119-122]. Unlike direct nerve stimulation, however, this orderly recruitment process (FT→ST) is speculative with the transcutaneous application of electrical current and appears to be influenced by a number of factors including the proximity of the motor axons to the applied electrodes and the branching pattern of these axons within the muscle [115, 123, 124].

NMES has been commonly used in numerous clinical settings as a rehabilitative tool in artificially activating skeletal muscle to attenuate atrophy and muscular dysfunction in pre- and post-operative orthopaedic patients [125-128] and in patients with peripheral or CNS damage or both [129, 130]. Significant atrophy and weakness of the quadriceps femoris muscle frequently occurs following knee ligament injury and anterior cruciate ligament (ACL) reconstruction [131]. Clinically, NMES has been used on ACL patients to minimize postoperative muscular atrophy and pain [127, 132-136]. Observed therapeutic benefits with NMES include, reduced strength losses in the early postoperative phase, increased gains in range of motion (ROM) around the knee joint, and enhanced rate of return to athletic activity [117, 127, 132-136]. Furthermore, NMES has also been applied to healthy active athletes as a means of increasing muscular mass and strength [137-141], and has been shown to minimize simulated microgravity-induced muscle atrophy and augment muscle strength [142-144].

Furthermore, according to Convertino et al [145], positive results obtained using NMES as a countermeasure to prevent disuse muscular atrophy may extend its benefits to reduce venous compliance in the lower

leg vasculature and subsequently the occurrence of orthostatic intolerance. Based on the data obtained from 30 days of bedrest, maintaining muscle size in the lower limbs by NMES provides structural support, limiting expansion of the veins and subsequently attenuating venous pooling during standing [144, 145]. Subjects with the largest reduction in muscle CSA and increased leg compliance reportedly fainted after standing post bedrest. Conversely, NMES treated subjects showed less reductions in muscle CSA with no change in leg compliance [144]. Therefore, maintaining leg muscle mass will likely attenuate high venous compliance and benefit postflight orthostatic stability. However, certain types of NMES may not be well tolerated by astronauts because high intensity depolarizing stimuli produces a strong muscle contraction and depending on its duration often produces discomfort. Thus, astronauts may not be willing to comply with such a countermeasure.

Therapeutic Electrical Stimulation (TES) is a type of NMES whereby the current intensity is at or below sensory threshold and does not evoke a visible muscular contraction. It is delivered continuously for 6-8 hours at night while the patient is asleep and has been suggested to be effective in the prevention and treatment of disuse atrophy in clinical settings [146]. TES is a successful modality that has been used to strengthen muscles in patients with cerebral palsy [147-149], myelomeningocoele [150], post-polio syndrome [151] and spinal cord injury [146].

In light of the clinical observations, it would seem reasonable to evaluate TES as a tool to control muscular wasting and dysfunction following exposure to non-weight bearing activity. The mechanism by which TES exerts its beneficial effects on muscular growth is unknown. NMES at frequencies ranging from 10-50 pps, delivered to the QFM with an intensity sufficient to produce mild contractions, has been shown to increase regional blood flow (RBF) and maintain it throughout the stimulation period [152]. TES, delivered

continuously over 6-8 hrs nocturnally, coincides with the diurnal variation in growth hormone (GH) secretion which peaks approximately 2 hours after sleep onset [153]. Thus, TES may exert its effect by increasing the delivery of GH to the affected muscles via the increase in RBF. GH enhances amino acid uptake and protein biosynthesis. Catabolic states, associated with the loss of body protein, frequently occur during injury and postoperative periods. GH therapy using recombinant human GH (rhGH) has been shown to significantly reduce protein catabolism in postoperative states and improve skeletal muscle strength [154, 155]. Therefore, it has been proposed that TES delivered nocturnally, may increase RBF and the transport of trophic factors to the muscle being stimulated. Another possible mechanism involves the stimulation of proprioceptive afferents via electrical stimulation provoking a trophic response in the muscle through sensory input to the motoneuron soma, where the production of trophic factors takes place. Most of the sensory input to the motoneuron is subthreshold (depolarization insufficient to generate an action potential), however, it may be adequate to induce alterations in the soma and play a role in the production of trophic factors and/or influence their rate of transport down the axon, eventually leading to the muscle being stimulated. Recent evidence suggests that activation of neural afferents via electrical stimulation can play a role in regulating the release of bioactive GH from the pituitary gland in rats [156]. High compliance (90%) with this therapeutic modality suggests it may be an attractive alternative to exercise regimens and supramaximal NMES in preventing microgravity-induced disuse muscle atrophy leaving astronauts with the maximal amount of time necessary to carry out their daily mission objectives.

Rationale For The Study

Ligaments of the knee joint are susceptible to injury and are very common in sports where knee twists are prevalent. Damage to the anterior cruciate ligament (ACL) occurs most frequently by either external rotation forces or hyperextension of the knee joint. Surgical intervention to repair the damaged ACL involves reconstruction of the ligament using biological grafts. The middle 1/3 patellar tendon graft is most commonly employed for ACL reconstruction and becomes fixed in a position via fibrous tissue. This particular biological graft heals within 6-8 weeks following surgery [131]. Profound atrophy and weakness of the quadriceps femoris muscle (QFM), related to disuse, can occur as early as the first few days following knee ligament injury [157-165] and ACL reconstruction [166-171]. In addition, the disuse atrophy observed may also be related to the reflex muscle inhibition of the QFM, caused by knee pain and chronic effusion around the joint [18]. This reflex inhibition of the QFM following injury or reconstruction of the ACL impairs a patients ability to voluntarily activate his/her QFM fully, and may contribute to the overall weakness and disuse atrophy of this muscle group [172, 173].

The marked atrophy and weakness of the QFM group following knee ligament injury and ACL reconstruction is applicable to the neuromuscular adaptations that arise in astronauts following exposure to prolonged weightlessness. A listing of microgravity-induced neuromuscular responses corresponding with observations reported in ACL patients is presented in Table XVII. Briefly, adaptations in QFM mass and structure in ACL patients are reasonably analogous to those observed following SF, including significant reductions in regional muscle volume, CSA, muscle fibre atrophy and alterations in fibre type composition. In addition, corresponding deficits of the knee extensors and altered neuromuscular function have been

SPACEFLIGHT RESPONSES	ACL Patient Studies	Details
MUSCLE MASS AND STRUCTURE		
Muscle Volume/CSA		
(-) QFM	(Lopresti, Kirkendall et al. 1988) (Rosenberg, Franklin et al. 1992) (Elmqvist, Lorentzon et al. 1988) (Elmqvist, Lorentzon et al. 1989) (Lorentzon, Elmqvist et al. 1989)	12% 13% (QFM & VMO) 6% 31% @ 20w 5%
(nc) BF	(Lopresti, Kirkendall et al. 1988)	
FCSA (VL biopsies)		
(-) type I	(Gerber, Hoppeler et al. 1985)	5%
(-) type II	(Lopresti, Kirkendall et al. 1988) (Baugher, Warren et al.1984)	~9% ~20% (VM)
(-) type IIa	(Gerber, Hoppeler et al. 1985)	4%
(-) type IIb	(Gerber, Hoppeler et al. 1985)	7%
Fibre Type Composition (VL biopsies)		
(-) type I	(Wigerstad, Grimby et al. 1988)	11%
(+) type IIa	(Arvidsson, Arvidsson et al. 1986)	5%
MUSCLE CONTRACTILE PROPERTIES		
(-) KE isokinetic strength	(Lopresti, Kirkendall et al. 1988) (Tibone and Antich 1988) (Seto, Orofino et al. 1988) (Wilk, Arrigo et al. 1992) (Rosenberg, Franklin et al. 1992) (Kannus 1988) (Kannus 1988) (Elmqvist, Lorentzon et al. 1988) (Dvir, Eger et al. 1989) (Elmqvist, Lorentzon et al. 1989) (Lorentzon, Elmqvist et al. 1989) (Itoh, Ichihashi et al. 1992	11-15% 13-17% 32-41% 24-31% 18% 14-19% 14-19% * * (21% pre-31% @20w post) 13-25% 25%,
(-) KE isometric strength	(Snyder-Mackler, Binder-Macleod et al. 1993) (Kannus 1988) (Itoh, Ichihashi et al. 1992)	40% 18% 14%,
(-) KE work capacity	(Wilk, Arrigo et al. 1992) (Kannus 1988) (Kannus 1988) (Elmqvist, Lorentzon et al. 1988) (Elmqvist, Lorentzon et al. 1989) (Lorentzon, Elmqvist et al. 1989)	25-30% 25% 22% * 27% pre -32% post 14w 20-29%
METABOLIC PROPERTIES (VM Biopsies)		
General		
(-) Capillary/Fibre ratio	(Gerber, Hoppeler et al. 1985)	5%
NEUROMUSCULAR ACTIVITY		
Electromyography		
(-) Neural drive	(Elmqvist, Lorentzon et al. 1988)	

* significantly less p<0.05

Table XVII: Studies involving ACL patients with neuromuscular responses corresponding with spaceflight-induced responses.

reported. Thus, ACL reconstructed patients provide a good model for studying treatments that can minimize such deficits in muscular function.

Rehabilitation following ACL reconstruction recently focuses on maximizing both the rate of recovery and return to full function by initiating motion very soon after surgery. These approaches now center on continuous passive motion exercises immediately following surgery within a restricted range of motion (ROM), strength recovery and neuromuscular re-education. Within 2 weeks following surgery, isometric contractions of the quadriceps femoris muscle are implemented to attenuate postoperative disuse atrophy and minimize strength losses [131]. Clinically, neuromuscular electrical stimulation (NMES) has also been used as a rehabilitative tool to minimize postoperative disuse atrophy and [127, 132-136]. Observed therapeutic benefits with NMES include, reduced strength losses in the early postoperative phase, augmented gains in ROM around the joint, and increased rate of return to athletic activity [117].

The following research was undertaken to address the issue of using TES as a countermeasure, by evaluating its effectiveness in minimizing muscular atrophy and dysfunction in post-operative orthopaedic patients. In this study, the efficacy of TES was determined by evaluating its effectiveness in comparison to that of a control group. In conjunction with a standard rehabilitation protocol, we proposed that TES would attenuate the underuse-related atrophy observed in the QFM and allow the patient to return to daily activities and athletics more rapidly. Further research then could be conducted during space travel in order to study the efficacy of TES as a countermeasure to prevent microgravity-induced muscle atrophy and dysfunction of the lower limbs. Unfortunately, due to the limited access of biological research on humans during long duration spaceflight, groundbase

studies on orthopaedic patients who are subject to underuse related atrophy, should be used as a precursor.

Study Objectives:

Our primary objective using TES was to conduct preliminary experiments to quantify stimulation parameters necessary to attenuate reductions in neuromuscular function. In view of the high cost and complexity of bedrest studies to simulate microgravity, we conducted a study on postoperative orthopaedic patients. The profound muscular atrophy and weakness of the quadriceps femoris muscle (QFM) following knee ligament injury is applicable to microgravity-induced underuse-related atrophy and provides a good model for studying treatments that can attenuate such deficits in muscular function.

Specific Aims:

The general purpose of this study was to determine if TES would prevent or attenuate the neuromuscular responses associated with underuse related muscle atrophy of the QFM following knee ligament surgery. A study on postoperative anterior cruciate ligament (ACL) patients permitted the examination of the effectiveness of TES. Comparisons were made between an experimental group, consisting of patients receiveing TES and a control group, consisting of patients not receiving any stimulation. The experimental paradigm was such that the involved QFM of each subject was stimulated using TES and its efficacy was determined by measuring its effectiveness in muscle strength and recruitment during isokinetic contractions.

Introduction

With the emergence of space travel, numerous physiological adaptations have been documented in weightless environments. The weightless environment of space decreases the activity and mechanical load of the muscles that are most important in moving and supporting body weight, in order to maintain erect posture. These muscles, primarily the lower extremity muscle groups, are termed weight-bearing muscles and in response to reduced usage, they begin to weaken and waste away, or atrophy. Changes include, decreases in muscle size [59, 60], breakdown of muscle protein [61], reductions in muscle strength [69, 70] and endurance [60] as well as changes in the types of fibres present in the muscles [60]. The muscular weakness and dysfunction arising from exposure to spaceflight is commonly referred to as microgravity-induced disuse atrophy.

None of these changes presents a problem to astronauts as long as they perform only light work. For astronauts, the problem becomes critical when they return to earth and the weakened muscles are once again subjected to the full force of gravity. In an emergency situation, individuals with weaker muscles would be less able to respond quickly or use physical strength.

At present, microgravity-induced disuse atrophy of the lower limb musculature continues to be a serious problem for astronauts following prolonged spaceflight. Countermeasures such as physical exercise [91] can help maintain muscle strength and function, however, exercise alone is insufficient to prevent excess muscle loss. Thus, there is a need to develop more effective solutions.

The present investigation was undertaken to evaluate the efficacy of therapeutic electrical stimulation (TES) on minimizing disuse muscle atrophy

and dysfunction in postoperative anterior cruciate ligament (ACL) patients. The ACL is frequently injured during sports participation. Significant atrophy and weakness of the quadriceps femoris muscle (QFM) group ensues following ACL reconstruction [131] and is applicable to the neuromuscular adaptations that arise in astronauts following exposure to prolonged weightlessness.

The purpose of this investigation was to conduct preliminary experiments to determine the stimulation paradigm most efficacious in minimizing microgravity-induced disuse atrophy in the lower extremities of astronauts. Using ACL reconstructed patients, provides a good model for studying treatments that can minimize such deficits in muscular function.

Treatment with TES was administered to the involved QFM 6-8 hours, 5 nights per week during the early postoperative phase of recovery, following ACL reconstruction with a patellar tendon graft. The efficacy of treatment was assessed in the QFM of the involved extremity by evaluating (a) isokinetic strength, (b) neural activation (iEMG), and (c) regional muscle volume during the initial 12 weeks of recovery.

Methodology

Subjects

Twenty four patients with a clinically proven ruptured ACL participated in this study. Patients were recruited prior to surgery, following a referral from the involved orthopedic surgeon at the Montreal General Hospital. Only patients who had elected for operative ACL reconstruction with a patellar tendon graft (Appendix I), and who did not have another injured ligament or previous injury to the knee, were included. Professional athletes and patients with neurological disorders or involvement of the contralateral knee were excluded. All surgical procedures were carried out by the same orthopedic surgeon. The mean time from the initial injury to surgery was 26.63 (3-120) months. The physical characteristics of patients are summarized in Table XVIII. Patients were randomly allocated to one of two groups described under «Design». All patients chosen for this study provided written informed consent approved by the Montreal General Hospital Research Ethics Committee (Appendix II).

Table XVIII: Physical characteristics of patients

Characteristics	TES (n=12)	Control (n=12)
Age (years)	28.45	26.73
Weight (kg)	74.31	72.66
Height (cm)	176.65	171.57

Values are means.

Design

The experimental paradigm was 12 weeks in duration following ACL reconstruction (Figure 1). It consisted of a preoperative orientation session, where patients were randomized into one of two groups (experimental and control) and familiarized with the testing equipment. Patients were evaluated preoperatively and postoperatively at 6 and 12 weeks. The experimental

group was administered TES during the 12 week trial and utilized a standardized rehabilitation protocol (Appendix III) in conjunction with TES. Each patient assigned to the experimental group was instructed on the proper use of the electrical stimulation unit and associated protocol (see below). The control group consisted of patients who did not receive any stimulation but underwent the same rehabilitation protocol.

EXPERIMENTAL PROTOCOL
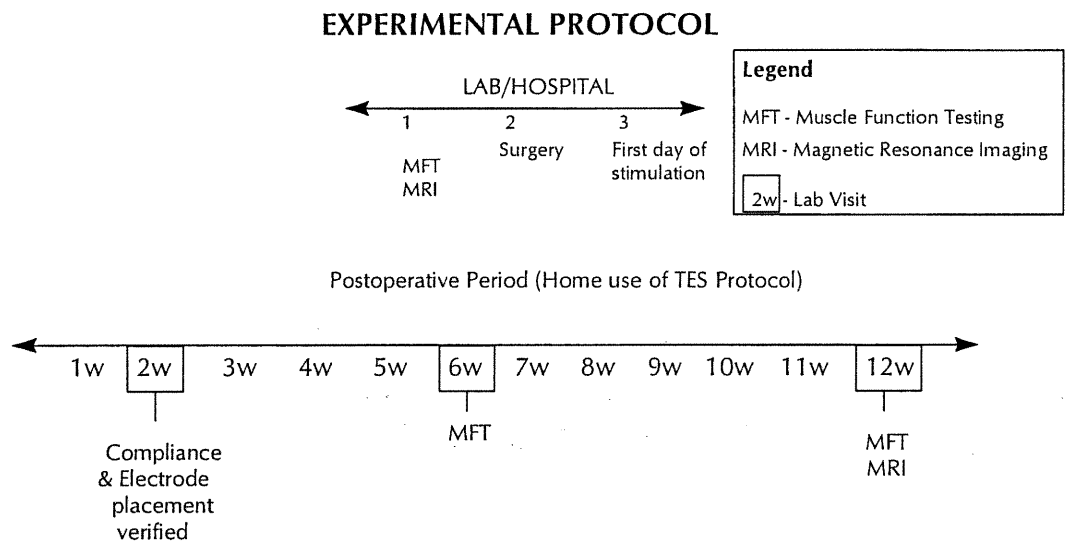
Figure 1: Experimental paradigm.

Muscle Strength Assessment

Strength was measured by quantifying the *in vivo* torque production of the knee extensor (KE) muscle group of both the involved and normal extremity during maximal voluntary contractions (MVC). Muscle torque production was measured using an isokinetic loading dynamometer (KIN COM 125 AP, Chattanooga Canada, Montreal, Quebec). Patients were seated on a specifically designed chair with their hip angle flexed 80 degrees, stabilized with restraining belts placed across the trunk and waist and thigh stabilizer bar placed over the thigh to isolate the quadriceps femoris muscle. The rotational axis of the

dynamometer was aligned with the axis of the knee joint (posterior to the lateral femoral condyle), and a shin pad was secured approximately 1 to 1.5 inches above the medial maleoli to allow free movement at the ankle. Each experimental procedure was preceded by a warm-up period to minimizes discomfort during the strength measurements and familiarizes the subject with the apparatus. The warm-up period consisted of:

1. Bicycle ergometer warm-up for ten minutes starting at 60 rpm then gradually raised to 90 rpm whilst maintaining a constant work level.
2. Five to ten sub-maximal repetitions and one maximal repetition at each angular velocity to be tested.

The uninvolved extremity was tested first. Maximal voluntary concentric contractions were performed at a constant velocity of 60 °/sec. and 180 °/sec. through a range of motion (ROM) of 75 ° (from 85 ° to 10 °), with 0 ° corresponding to the full extension of the knee. Each test consists of several contractions, interspersed by a 1 minute rest period between each contraction and 2 minute rest period between respective speeds, until the two successive torque curves are superimposed upon one another signifying the patients true MVC [199].

All dynamic contractions were preceded by a maximal pre-loading static contraction with a duration of 1.5 seconds. The purpose of this static pre-loading was to allow for the contractile tension to rise before movement was permitted, thus eliminating its influence on the early part of the torque curve [200]. Instructions to each patient were standardized with visual cues and verbal feedback was given to encourage maximal effort. All torque measures during voluntary contraction were gravity corrected.

Electromyographic Assessment

EMG activity from the vastus medialis (VM) and vastus lateralis (VL) were simultaneously recorded with the force and angle signals. After careful skin preparation, surface bi-polar electrodes (DE-02 Differential EMG electrodes, Delsys Inc., Wellesley, MA) were placed over the mid-line of the muscle belly between the motor point and the myotendinous junction (Appendix IV). In this location, the EMG signal with the greatest amplitude is detected [201].

Differential Electrode Configuration and Data Acquisition

Detection surfaces consisted of two parallel bars, each 1 cm long, 1-2 mm wide, spaced 1 cm apart, oriented perpendicular to the length of the muscle fibers. These electrodes were connected to a miniature pre-amplifier and then to a connector box. A shielded cable joined the connector box to the data acquisition card (Bagnoli-8 EMG Data Collection System, Delsys Inc., Wellesley, MA). For a schematic of the instrumentation used for the data acquisition system see Appendix V.

Raw EMG signals were then amplified and band pass filtered (20-500 Hz) with the Delsys Isolated Myoelectric Amplifier. EMG signals from two channels were sampled at a rate of 4 kHz by the analog-to-digital converter of the Dell Optiplex 486 computer over the signal range ± 5 V. A computer program developed from the NI-DAQ library version 4.4 (National Instruments, Austin, TX) was used for data acquisition and on-line data analysis. For each trial, data acquisition was triggered using the velocity signal transmitted by the lever arm at the start of movement.

Processing of Torque and EMG Data

All data were analyzed from the MVC of the QFM of both extremities for each of the two speeds. Peak torque was calculated as the highest point generated on the torque curve measured in Newton-meters (Nm). Total work was defined as force times distance, and it is the entire area under the torque curve measured in Joules (J). Mean power was calculated as total work defined by the time for the MVC, measured in Watts (W).

Digitized EMG data was processed with a 1024 point Fast Fourier Transform (FFT) to obtain the power density spectrum of the signal. A series of 40 and 8 consecutive spectral windows were averaged over the entire range of movement and used to calculate the median power frequency (MED), measured in Hz, at 60°/sec. and 180°/sec., respectively. Quantification of the EMG signal was accomplished by calculating the integration (area under the curve) of the full-wave rectified signal generated during the MVC, measured in Vsec. (Maximal iEMG). Mean knee extension iEMG ($iEMG_{QFM}$) was calculated by adding the mean values for each muscle and dividing by two:

$$iEMG_{QFM} = [\text{Vastus Lateralis iEMG} + \text{Vastus Medialis iEMG}] / 2$$

Stimulation Procedure

During the preoperative orientation session, patients assigned to the experimental group were instructed on the use of the stimulator and application of the TES protocol. Stimulation parameters for the nocturnal implementation of TES are listed in Appendix VI.

The "One2One" (Mayatek, Toronto, Ontario) portable battery-powered neuromuscular electrical stimulator was used to stimulate the QFM group of the involved extremity. Two self-adhesive bipolar electrodes conducting the stimulating current were placed on the skin overlying the

vastus medialis (VM) and rectus femoris (RF) muscles of the involved extremity according to Appendix VII. The positions of these electrodes were marked using a non-toxic water-soluble marker so that the patient was able to reproducibly apply them to the correct positions at home. With the electrodes in place, the patient was instructed on the proper use and maintainance of the equipment and the appropriate stimulus intensity (patient setting) was determined according to Appendix VIII. All patients were required to setup and activate the stimulators, with the electrodes in place before leaving the laboratory. Following surgery, once at home, the patient began the treatment for the selected duration and recorded his/her subjective responses to the electrical stimulation as outlined in the «Patients Stimulation Log» (Appendix IX). Each stimulator comes equipped with an internal compliance timer in order to track patient use.

At two weeks postoperatively, the patient returned to the laboratory for a follow-up visit. At that time, the compliance meter and proper electrode placement was verified. At each postoperative testing period the patient was required to bring the stimulator in order to verify the compliance and collect the log sheets.

Magnetic Resonance Imaging (MRI)

Transverse MRI images were processed from a point 26 cm below the anterior superior iliac spine (ASIS) inferior to the knee centre. Slices were 10 mm thick, with a 10 mm gap between slices. After a coronal localizer three sets of scans were obtained: an axial T1, axial FSE and an axial fast stir. The MRI data were electronically transferred from the MGH to our laboratory where the axial T1 images were analyzed using the Osiris (version 3.2) software program for analysis. Muscle was delineated from bone and adipose

tissues. Muscle volume measurements were computed as cumulative areas of sequential slices from the scanned region - expressed separately as volumes of total muscle (QFM) and specifically of the VM.

Statistical Analysis

Statistical analysis of data was prepared by James A. Hanley Ph.D for the Canadian Space Agency [202]. Pre- and postoperative data were statistically compared by means of a 2-way ANOVA, with different groups (TES vs. Control) and time (repeated measures) as the main factors. The null hypothesis was that stimulation had no effect and that no difference was present between control and stimulation groups. Significance level will be determined at $p < 0.05$ level of probability.

Data Reduction

The first task was to bring together the 6 observations on a patient, i.e. for the 2 legs, each of three times. The considerable variation from patient to patient in individual baseline values, would obscure any treatment effect. Therefore, we standardized the measurement at 0, 6, and 12 weeks on the involved leg to the 0 week measurement on the uninvolved leg. Therefore, we divided the value for the involved leg by the value for the uninvolved leg.

The preoperative values for the standardized value for the involved leg still varied somewhat from person to person (for example, for the force variable PT_ROM, the pre-op values for the 12 persons at speed 60 in the control group varied from 0.65 to 1.06, mean 0.87, SD 0.14). This may reflect that the involved leg in some patients may have been the "dominant" leg, whereas in others it may not have been. However, our analysis (see below) uses the ***within patient*** change over time, so that patient to patient variation

at baseline is effectively removed. The same standardization procedure was used for each of the Force, VL-iEMG, VM-iEMG and MRI variables analyzed, with MRI measures being taken only twice at 0 and 12 weeks.

Descriptive Summary Statistics

The average values of each of the Force, VL-iEMG and VM-iEMG variables were calculated for each speed at 3 time points (0, 6 and 12 weeks). The average values of MRI variables were calculated only at two time points (0 and 6 weeks).

Formal Tests of Significance

Overall tests of statistical significance for the difference between groups in the time-course of each variable were performed using the «PROC MIXED» procedure in SAS [203].

«Group» and «time» were treated as categorical variables, and the focus was on *the group by time interaction*. Lack of interaction would signify that the two time courses are *parallel*, while the existence of an interaction would mean the two were '*non-parallel*' in some sense. When we have three time points, the test statistic for the interaction has 2 degrees of freedom (2-1)(3-1) and measures all possible *non-parallel* patterns.

A more sensitive and focused test was carried out by concentrating on the expected or hoped for changes in the time course produced by the treatment. These were a lesser decline from 0 to 6 weeks in the treatment group relative to the control group. This was assessed using the contrast:

[week 6 – week 0](Treatment)- [week 6 – week 0](control),

so that a *positive* quantity reflects favorably on treatment e.g. if measurements were 0.53 in week 6, and 0.81 in week 0, in the control

group, then the change would be −0.28. Correspondingly if the values were 0.58 and 0.82 in the treatment group, then the change would be −0.24. The difference of the two, -0.24 and -0.28, is +0.04. One reason to concentrate on the 0 - 6 week change is that, once patients were allowed to use the involved leg freely (at about 6 weeks), each patient would be engaged in a different (and possibly considerable) amount of exercise, therefore obscuring the (smaller) effects of treatment.

Shallower decline by the 6 week, and greater recovery by the 12 week in the treatment group to the control group. This was assessed using the contrast of quadratic effect:

[week 6 − average(week 0 and week 12)](Treatment)

Minus

[week 6 − average(week 0 and week 12](Control),

again so that a ***positive*** quantity reflects favorably on treatment. For example, suppose that 3 measurements were 0.87, 0.38 and 0.53 for a patient in control group at 0, 6, and 12 weeks. The average (mid value) of 0.87 and 0.53 is 0.70, whereas in fact, the 6 week mean was 0.38, i.e. a «dip» of −0.32. In contrast, suppose the corresponding values for a patient in the treatment were 0.87, 0.47 and 0.59. Then the mid value of 0.87 and 0.59 is 0.73, whereas, the 6 week mean is 0.47, a lesser «dip» of −0.26. Thus, the difference of the two «dips» is −0.26-(-0.32) =0.06, in favour of treatment.

The MRI measurements were only made twice, at 0 and 12 weeks. For these, we used the same contrast as the above, but with week 12 instead of week 6. An increase in the MRI measurement values from preoperative to postoperative in the treatment group to control group would show the effect of treatment.

The estimates, their standard errors, student t-values, and 2-sided p values were obtained by defining each estimate as a contrast, and treating each patient (nested within group) as a random effect.

Results

Analysis of variance demonstrated significant differences between pre and postoperative values when only the effect of surgery was taken into account. With regards to the efficacy of treatment on these postoperative changes, tendencies for an effect were observed; however, these did not reach statistical significance. Standardized (see Methodology) values are expressed as the % difference from preoperative to postoperative measurements ± standard error. Therefore, results exhibiting a p value less than or equal to 0.1 are presented and marked with an asterisk (*) and statistically significant values of p<0.05 and p<0.01, will be marked with two (**) and three (***) asterisks, respectively. These data are presented in Appendix X.

Part I: Postoperative Effects of Surgery

Isokinetic Test Measurements

Peak Torque

At 6 weeks postoperatively the mean quadriceps femoris muscle (QFM) peak torque (PT) deficit of the operated knee (Figure 2) was 49.0±4.0% at the speed of 60°/sec. and 47.4±6.1% at the speed of 180°/sec. (p=0.000). At 12 weeks postoperatively, the deficits were not as severe, exhibiting a deficiency of 33.7±4.0% at 60°/sec. and 29.7±6.1% at 180°/sec. (p=0.000).

Figure 2: Postoperative effect of surgery on mean involved QFM isokinetic peak torque

*** Significant from preoperative measurements (p<0.01)

** p<0.05

Time To Peak Torque

At 6 weeks postoperatively, time to peak torque (TTPT) was increased by 81.8±41.8% at 60°/sec., but this was not statistically significant (p=0.063) (Figure 3). No significant differences were demonstrated at 180°/sec., nor at 12 weeks at either speed.

Total Work

The mean total work deficit of the involved QFM (Figure 4) at 6 weeks postoperatively was 34.7±6.3% at 60°/sec. and 43.5±8.3% at 180°/sec. (p=0.000). At 12 weeks postoperatively, the deficiency lessened, with deficits of 18.8±6.3% (p=0.007) and 25.5±8.3% (p=0.006) at 60°/sec. and 180°/sec., respectively.

Mean Power

At 6 weeks postoperatively, the mean power deficit for the involved QFM was 35.8±6.4% at 60°/sec. and 44.1±8.4% at 180°/sec. (p=0.000). Deficits at 12 weeks postoperatively were reduced to 20.3±6.4% and 28.9±8.4% at 60°/sec. (p=0.005) and 180°/sec. (p=0.002), respectively (Figure 5).

Integrated Electromyographic Activity and Power Density Frequency Spectrum

Individual Muscle Activities

The mean maximal iEMG activities for the Vastus Lateralis (VL) and Vastus Medialis (VM) muscles are shown in Figure 6. At 6 weeks
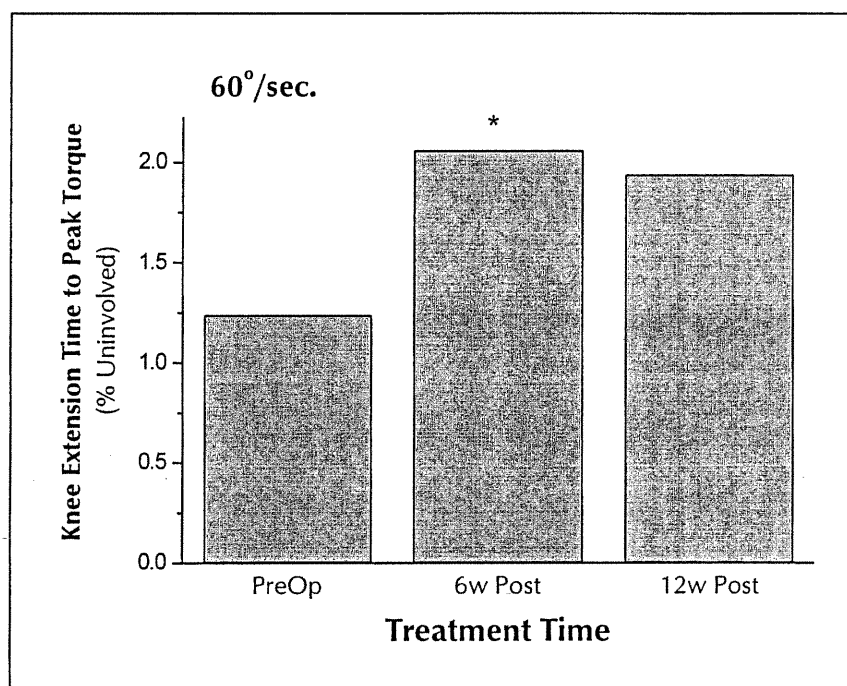
Figure 3: Postoperative effect of surgery on mean involved QFM time to peak torque
  * Not significant from preoperative measurements (p<0.1)
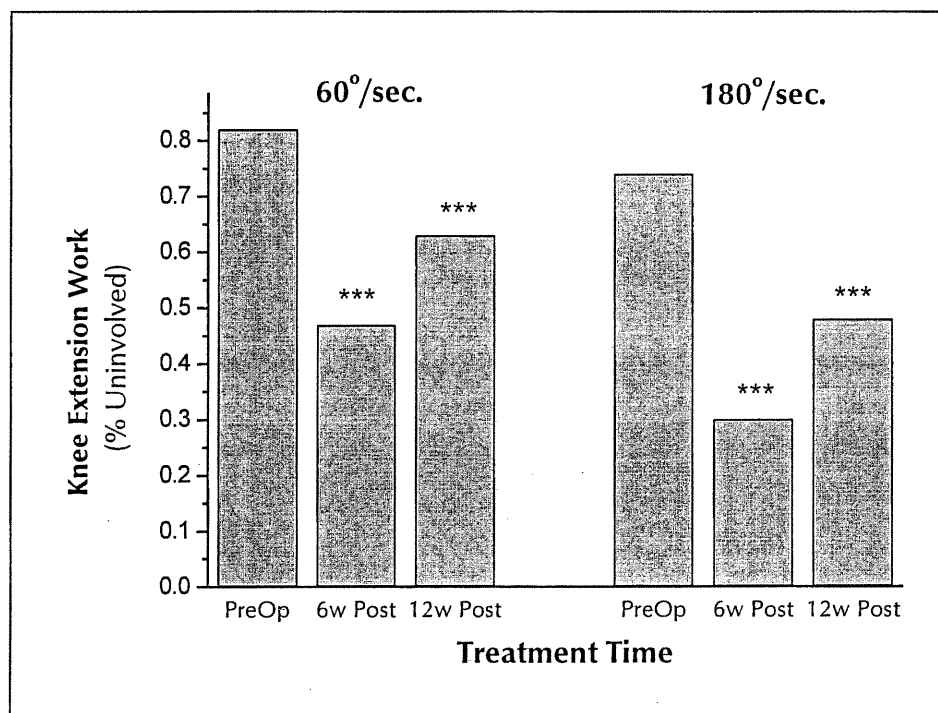
Figure 4: Postoperative effect of surgery on mean involved QFM isokinetic total work

$***$ Significant from preoperative measurements ($p < 0.01$)
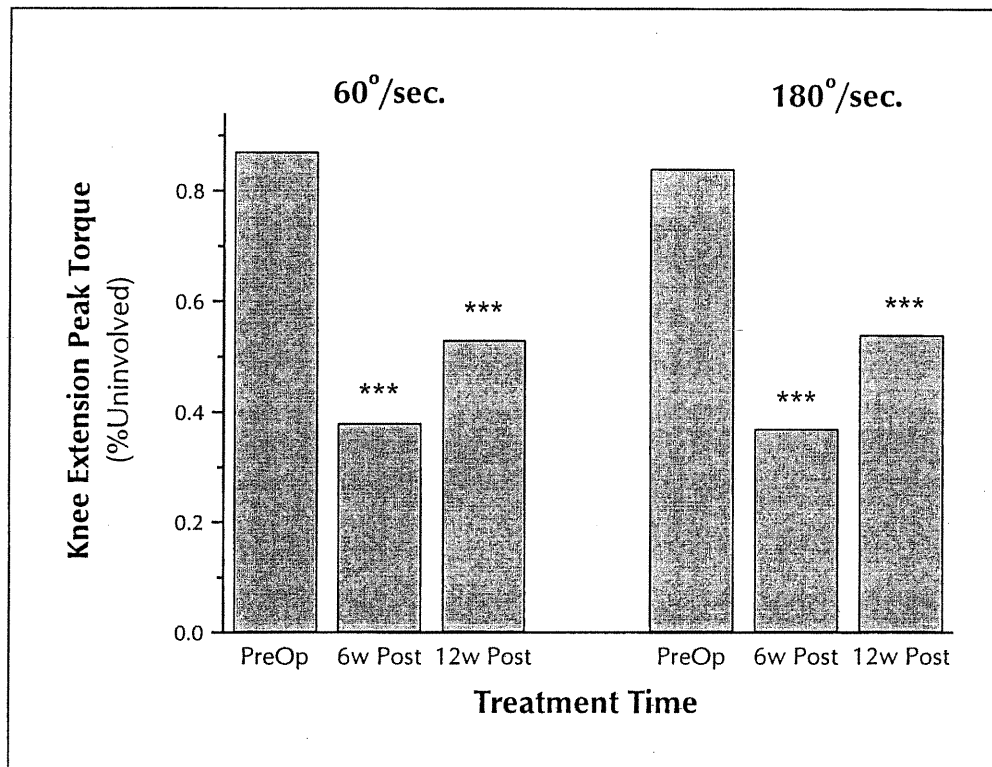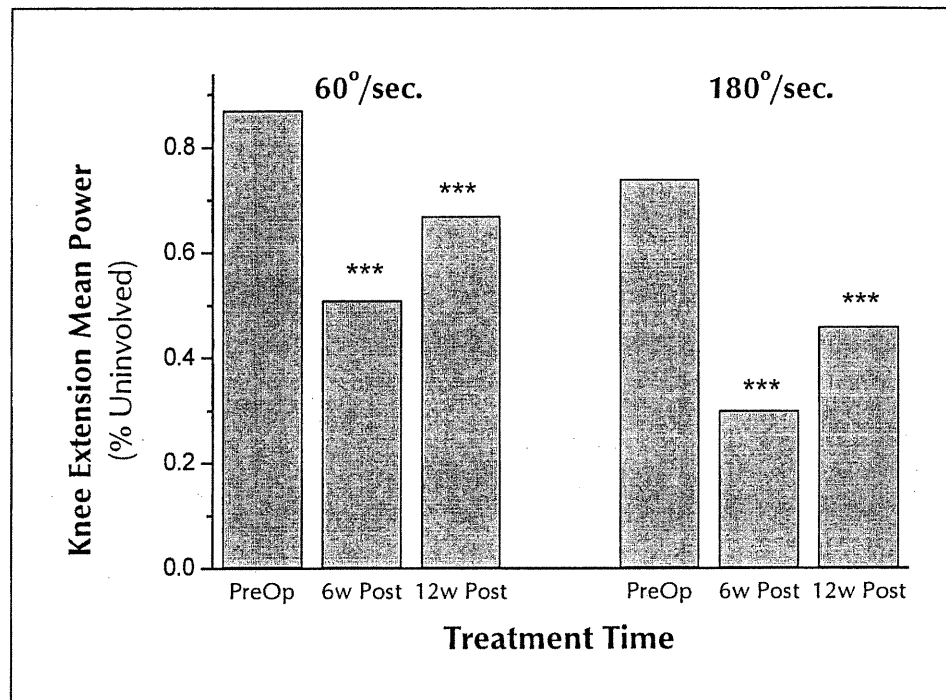
$**$ $p < 0.05$

Figure 5: Postoperative effect of surgery on mean involved QFM isokinetic mean power

*** Significant from preoperative measurements (p<0.01)

** p<0.05

Figure 6: Postoperative effect of surgery on mean maximal iEMG activity of the involved VL and VM muscles

*** Significant from preoperative measurements ($p < 0.01$)

** $p < 0.05$

postoperatively, mean maximal iEMG activity for the VL muscle was reduced by 38.2±4.9% and 48.5±9.9% at 60°/sec. and 180°/sec. (p=0.000), respectively. A slight recovery of maximal iEMG activity was observed at 12 weeks postoperatively, exhibiting decrements of 27.9±4.9% at 60°/sec. (p=0.000) and 20.7±9.9% at 180°/sec. (p=0.048).

In contrast to the VL muscle, mean maximal iEMG activity for the VM muscle at 60°/sec. featured no significant changes from preoperative measurements and remained constant throughout the 12 week postoperative period. However, at 180°/sec., the VM maximal iEMG activity exhibited a significant improvement of 31.3±14.9% between 6 and 12 weeks postoperatively (p=0.047).

Mean Knee Extension iEMG

A significant reduction in the average mean knee extension iEMG (EMG_{QFM}) was observed at 6 weeks postoperatively with deficits of 23.7±6.5% at 60°/sec. (p=0.001) and 33.1±10.0% at 180°/sec. (p=0.003). At 12 weeks postoperatively, the deficiency at 60°/sec. persisted but recovered slightly with only a 13.5±6.5% decline in $iEMG_{QFM}$ (p=0.048). However, at 180°/sec., the deficiency recovered and was no longer statistically significant at 12 weeks postoperatively (Figure 7).

Median Power Frequency

The average median power frequency (MED) of the power density spectrum generated for the VL muscle was significantly reduced by 15.1±4.3% and 19.0±6.1% at 6 weeks postoperatively during maximal voluntary contractions at 60°/sec. (p=0.002) and 180°/sec. (p=0.005), but
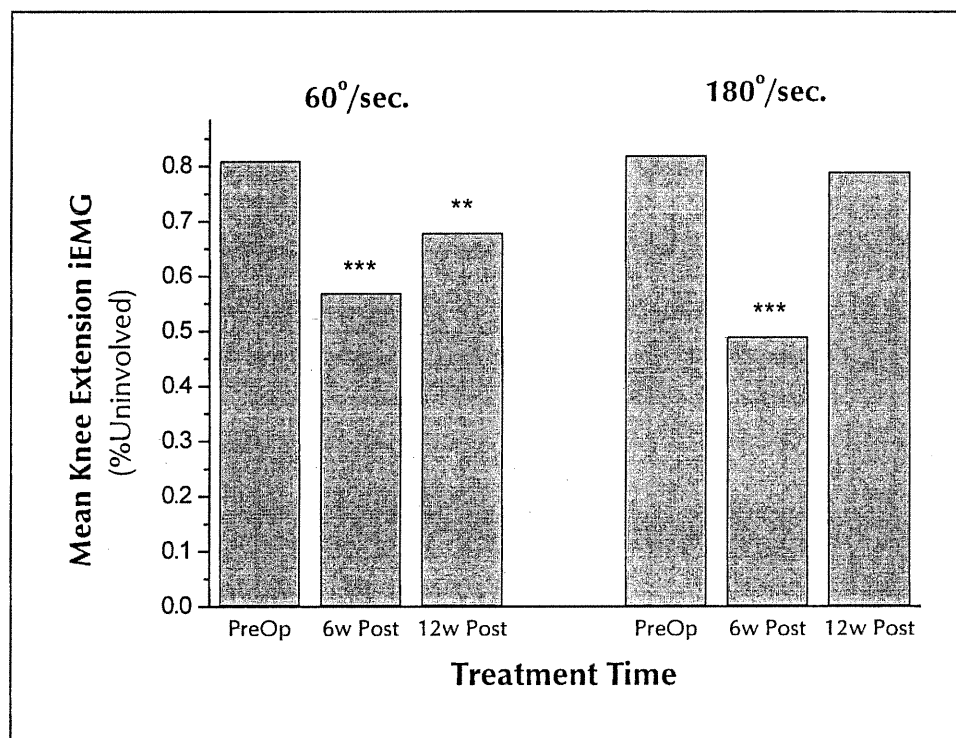
Figure 7: Postoperative effect of surgery on mean involved QFM iEMG

*** Significant from preoperative measurements (p<0.01)

** p<0.05

returned to within preoperative values by 12 weeks postoperatively (Figure 8).

In contrast, at 6 weeks postoperatively, the reduction in average MED generated from the VM muscle under the same isokinetic conditions was double that of the VL at 60°/sec., exhibiting a decrement of 30.8±7.8% (p=0.001) whereas a decline of 28.3± 10.0% was observed at 180°/sec. (p=0.01). Unlike the VL, at 12 weeks postoperatively, this decline in MED persisted with 18.2±7.8% and 21.0±10.0% reductions observed at 60°/sec. (p=0.028) and 180°/sec. (p=0.048), respectively.

Muscle Volume Measurements

Patients demonstrated no mean changes in the measurement of the overall QFM volume at 12 weeks postoperatively. In contrast, the mean individual VL muscle measurements showed a decrease of 23.1±10.8% at 12 weeks with a tendency toward significance (p=0.07) (Figure 9).

Part II   Effect of Treatment on Postoperative Changes

Isokinetic Test Measurements

Analysis of variance showed no significant differences between the group means of force deficits namely, PT, work and mean power when measured at either speed both at 6 weeks and 12 weeks postoperatively. However, in contrast to the control group, the experimental group undergoing treatment with TES was able to maintain their mean TTPT values throughout the postoperative period (Figure 10). This signifies a positive effect of treatment with a tendency towards significance (p=0.05).
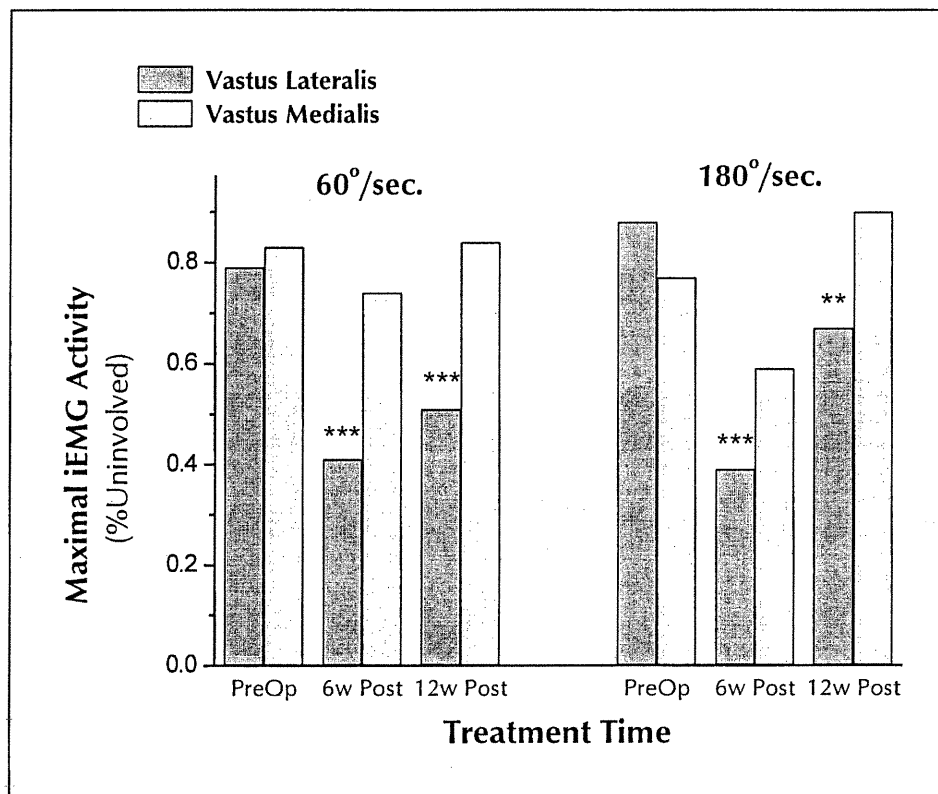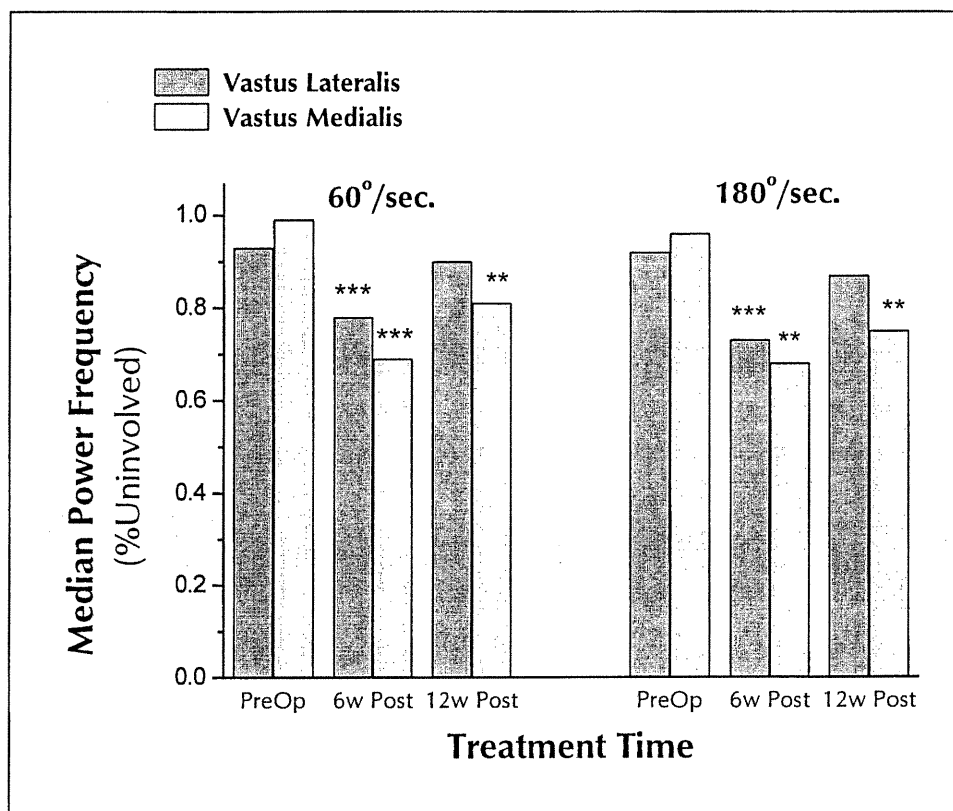
Figure 8: Postoperative effect of surgery on mean MED of the involved VL and VM muscles

*** Significant from preoperative measurements (p<0.01)

** p<0.05
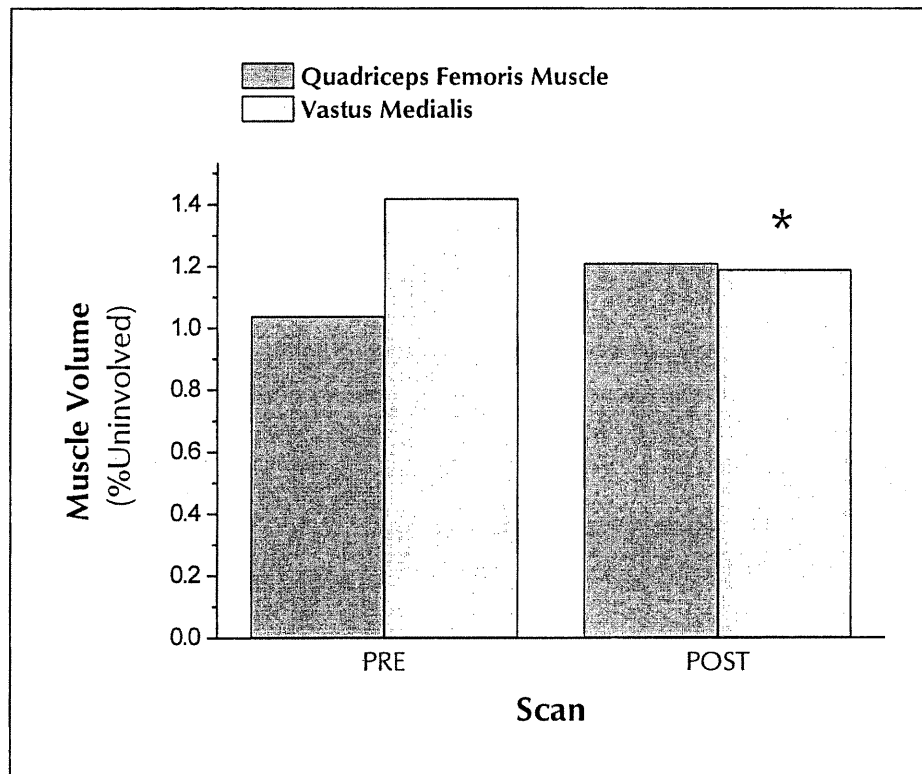
Figure 9: Postoperative effect of surgery on mean involved QFM and VM muscle volume

* Not significant from preoperative measurements (p<0.1)
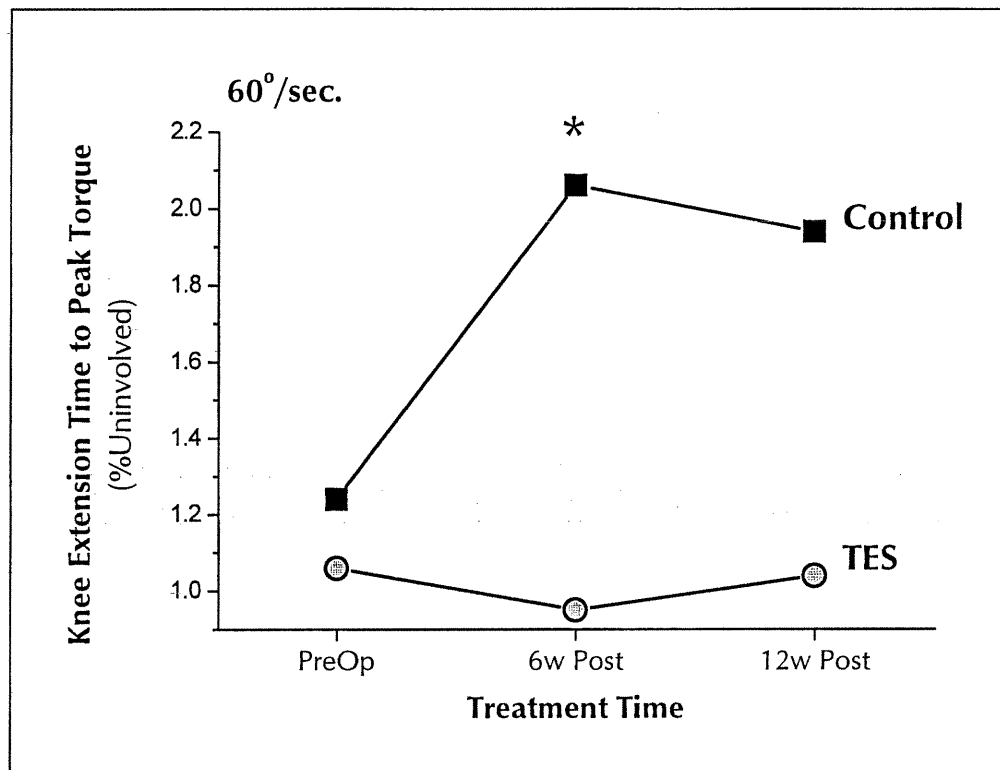
Figure 10: Postoperative effect of treatment on mean involved QFM time to peak torque

* Not significant from preoperative measurements (p<0.1)

Integrated Electromyographic Activity and Power Density Frequency Spectrum

No significant differences were observed between group means at 6 weeks postoperatively, in terms of maximal iEMG activity of the VL muscle during the production of a MVC at 60°/sec. (Figure 11). At 180°/sec, however, the experimental group demonstrated less of a decline in mean maximal activity of the VL muscle, when compared with the control group, but this was not significant (p=0.09)

In direct contrast to the control group, patients in the experimental group exhibited a strong deficit in the mean maximal iEMG activity of the VM muscle, at 6 weeks postoperatively, during the production of a MVC at 60°/sec. (Figure 12). This deficit translates into a 25.0±13% difference in the mean maximal VM iEMG activity between patients in the control and experimental group; again this difference was not significant (p=0.06). No significant differences were detected in the mean maximal iEMG activity of the VM at 180°/sec when comparisons were made between groups. At 12 weeks postoperatively, no significant differences were found between group means when the maximal iEMG activity of the VL or VM muscles were compared at either speed.

Muscle Volume Measurements

Patients in the experimental group demonstrate no significant effects or tendencies toward an effect of treatment with regards to overall QFM volume and VM muscle volume measurements at 12 weeks postoperatively.
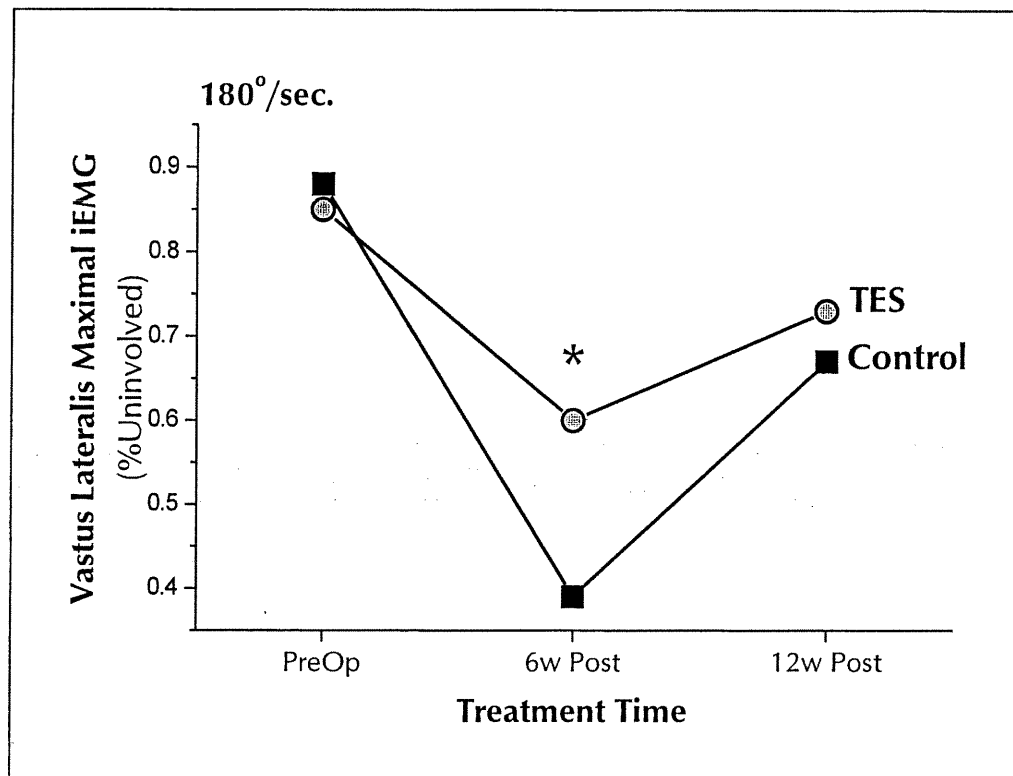
Figure 11: Postoperative effect of treatment on mean involved maximal VL iEMG activity

* Not significant from preoperative measurements (p<0.1)
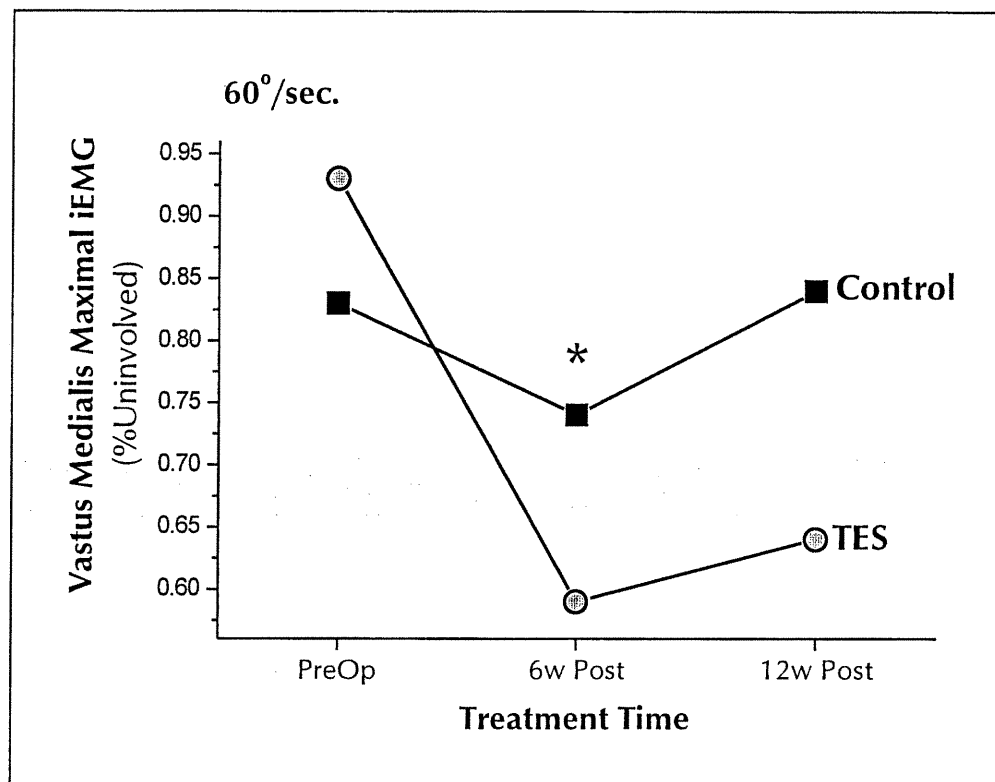
Figure 12: Postoperative effect of treatment on mean involved maximal VM iEMG
activity

* Not significant from preoperative measurements (p<0.1)

Discussion

How do our results following ACL reconstructive surgery compare with the previous literature on ACL reconstructed patients?

Comparisons with the previous literature were restricted to patients that were not submitted to a postoperative immobilization period following ACL reconstruction, whenever possible. We felt that comparisons with our patients to studies involving prolonged immobilization would be inappropriate, since the detrimental effects of joint immobilization namely, joint stiffness and severe atrophy and weakness of the thigh musculature [204], would certainly introduce confounding variables, making possible comparisons with patients in this study, highly questionable.

Data gathered from the "Patients Stimulation Log" of 12 patients in the experimental group, were totaled and averaged. Stimulation was applied to their affected QFM an average of 461.4 total hours over the 12 week postoperative period. Average number of hours of stimulation per day was 7.69 with an average intensity of 5.47 mA.

Isokinetic Test Measurements

The present study confirms that patients with an ACL deficient knee exhibit weakness in the QFM. Preoperatively, bilateral strength comparisons between the involved and uninvolved QFM are in agreement with literature, defining ratios of 80-89% and 70-79% as indicative of minimal and moderate deficiency, respectively [205, 206]. Please refer to Appendix X.

Postoperatively, the results of this study demonstrate that the isokinetic measurements of the QFM exhibit persistent deficits in PT, work and mean power for up to 12 weeks following reconstruction of the ACL. At 6 weeks, mean deficits in PT ranged between 47-49%, depending on the

speed of contraction. At 12 weeks, these mean PT deficits significantly improved, ranging from 29.7-33.7%. These results are in close agreement with Shelbourne et al. [207] who reported a ~41% and ~31% deficit in QFM isokinetic PT at 180°/sec., 6 weeks and 12 weeks following ACL reconstruction, respectively. With respect to the interval between 6 and 12 week postoperative evaluations, patients in our study exhibited a better recovery in comparison to the latter investigation by Shelbourne et al. [207].

In our study, postoperative mean total work deficits of the involved QFM, relative to the uninvolved muscle, were in accordance with previous studies which reported similar torque deficits in the QFM after ACL reconstruction [169, 208, 209]. At 12 weeks postoperatively, our patients exhibited a 25.5% reduction in mean total work capacity at 180°/sec.. Similarly, deficits of 30% [169], 25.2% [209] and 20% [208] have been reported at 12 weeks, 15 weeks and 16 weeks following ACL reconstruction, respectively.

Postoperative mean power deficits persisted over the 12 weeks with a 28.9% reduction observed at 180°/sec.. This result is highly comparable with the 29% deficit in mean power reported by Wilk et al. [169] during a 12 week follow-up testing at 180°/sec. following ACL surgery.

Integrated Electromyographic Activity

Mean standardized iEMG values of the VM and VL muscles provide a good representation of the level of activation in these muscles during the performance of a MVC at selected speeds. For the VL muscle, a significant decline in its activity persisted throughout the 12 week postoperative period, ranging from 38.2% and 48.5% at 6 weeks to 27.9% and 20.7% at 12 weeks during maximal contractions at 60°/sec. and 180°/sec., respectively. Unlike

the VL muscle, a marked increase in maximal iEMG activity of 31.3% was observed postoperatively in the VM muscle from 6 to 12 weeks at 180°/sec., bringing the activation of the VM to within 90% of the uninvolved VM muscle. Unfortunately, no comparisons can be made with previous findings regarding changes in the activation of individual muscles in ACL reconstructive patients.

Earlier studies reporting deficits in maximal iEMG activities in ACL patients are limited to observations in patients sustaining an injury to the ACL, without surgical intervention. These findings are in close relation to the observed iEMG deficits in the injured limb of patients with an ACL tear, where the activity of the VL and VM muscles were reportedly reduced by 19.2% and only 0.6%, respectively, relative to the non-injured limb [161].

In our study, the significant decline in mean knee extension iEMG activity, measured at 6 weeks postoperatively, persisted over the following 6 weeks during the production of a MVC at 60°/sec.. However, no statistical deficits were observed at 180°/sec. by 12 weeks. This was in contrast to Elmqvist et al. [161], where a 13% deficit remained in the mean iEMG activity of the injured QFM, when the individual iEMG values for the rectus femoris (RF), VM and VL muscles at 180°/sec., were taken into account.

According to Sale et al. [210], maximal iEMG is a well established measure of the amount of electrical activity produced within a muscle by representing the individual activation of muscle fibers of recruited motor units during the production of a MVC. Reduction in the maximal iEMG activity of individual muscles can reflect an inability of that muscle for complete activation and point to a disruption in the neuromuscular mechanism [173]. If maximal activation is reduced, this suggests a diminished capacity for the proper utilization of motor units, leading to reduced motor unit excitation and resulting in a diminished performance of the muscle [173].

Altered utilization of motor units in the VM and/or VL muscle can arise from the reflex inhibition of the QFM, after injury or reconstruction of the ACL. Reflex inhibition arises from the receptor afferents located in the knee, which provide an altered sensory feedback to the motoneuron pool in the spinal cord and consequently leads to a decreased neural input or neuromuscular drive to the muscle [18]. This reduction in neuromuscular drive to the muscle may, in part, account for the deficits in the isokinetic performance of the QFM in patients following ACL reconstruction.

Power Density Frequency Spectrum

The shift in MED of the VL and VM muscles over the early postoperative phase may imply that a significant proportion of type I fibres over type II fibres were preferentially recruited during the isokinetic maneuver. Previous findings have indicated that the MED is a reflection of the percentage of muscle fibres recruited, where a higher percentage of type II fibres generates a higher MED and a higher proportion of type I fibres generates a lower MED [211].

Can a MED shift to lower values predict the occurrence of reflex inhibition during a MVC? Altered motor unit utilization may be responsible for the marked reduction in iEMG activity and partly responsible for the subsequent deficits in isokinetic performance. Since type I fibres are more easily recruited than type II fibres during voluntary contraction, a reduction in type II fibre involvement during a MVC, as reflected in the MED shift, would be expected in a situation of reduced neural input and result in the diminished neuromuscular performance of that muscle [212] Therefore, in the context of a MVC, the MED shift to lower values can highlight the occurrence of reflex inhibition.

Muscle Volume Measurements

No significant differences were observed between preoperative and postoperative measurements of the overall QFM and individual VM muscle volumes. However, there was a tendency for a decrease in the postoperative VM volume. Previous findings in thigh muscle volumes on ACL reconstructed patients were not found, however, this decline in muscle volume would signify a degree of atrophy present in the VM, but not statistically significant. Between 5-31% atrophy has been reported in the literature following ACL injury and reconstruction [161, 163, 164, 166, 170].

What are the effects of treatment with TES during the early postoperative phase of recovery following ACL reconstruction?

The aim of the present study was to compare the effect of TES coupled with a standard rehabilitation protocol with patients following the rehabilitation protocol exclusively. Our results indicate that there were no significant differences between the experimental and control groups. We wanted to see if TES would attenuate any of the effects of surgery as previously discussed. TES, in addition to rehabilitation, provided no benefit to the subjects in minimizing the effects of surgery measured in this study, during the early postoperative phase of recovery (0-6 weeks) following ACL reconstruction. Of the numerous outcome measurements undertaken during this investigation to test the possible benefits of TES, only TTPT and mean iEMG activities of the VL and VM muscles exhibited a tendency for a treatment effect, during the early postoperative phase.

Mean TTPT values were maintained throughout the treatment period in comparison to the control group, approaching significance at 6 weeks postoperatively with a p-value of 0.05. TTPT is indicative of the type II/type I fibre ratio [213]. Force-time characteristics of the QFM likely reflect the fibre composition of the muscle, since a reduction in TTPT has been observed following significant gains in force generating capacity in power athletes and after strength training [213]. A lack of change in this parameter, as noted in our study, may suggest that treatment with TES is efficacious in maintaining the neural input to the muscle as well as the time for maximal activation.

In comparison to the control group, the effects of surgery on the decline of the maximal iEMG activity of the VL muscle was attenuated by TES, showing a tendency for a positive effect of treatment, during the early postoperative period. Contrary to this finding, however, maximal iEMG activity of the VM muscle in the experimental group was observed to

decrease, unlike the control group, where the maximal iEMG activity of the VM muscle was maintained throughout the initial postoperative stages of recovery. This finding may signify a tendency for a negative effect of treatment, however, it is more likely explained by a reduction in neural drive to the muscle due to the presence of reflex inhibition [173, 214], commonly observed during the early postoperative phase [135].

The literature concerning the effects of NMES in patients during the early postoperative phase following ACL reconstruction, is quite limited and inconsistent. The rationale behind the use of NMES protocols during the initial stages of recovery lies in the inability for intensive volitional exercise of the muscle for the prevention of atrophy, since muscles around the joint are significantly restricted [204]. NMES has been preferentially shown to activate type II fibres [215, 216] and selective atrophy of type IIB fibres have been reported in the literature following ACL reconstruction [171]. Furthermore, because type II motor units are only activated at high levels of voluntary contraction, selective atrophy of these fibres may be manifested in strength deficits during the performance of a MVC. Therefore, NMES may be a useful treatment for patients who are weak. It is mainly for this reason that NMES has been considered as an effective supplementary tool to the standard rehabilitation of inactive muscle groups [115, 214].

NMES has been shown to be effective in preventing deficits in strength, muscle mass and oxidative capacity of the QFM following knee ligament surgery [127, 132, 133, 136], while others have reported no significant differences in strength gains between groups training with voluntary contractions and those training with NMES [125, 136, 217, 218]. One must keep in mind that the inadequate standardization of experimental procedures, evaluation methods and stimulus protocols makes comparing these studies and drawing accurate conclusions concerning the utilization of NMES difficult.

Following ACL reconstruction, treatment with NMES protocols during the early postoperative phase of recovery is limited to relatively few studies

in the literature [134, 136, 218, 219]. Delitto et al. [134] compared the effectiveness of NMES with voluntary exercise in minimizing postoperative strength deficits. The experimental protocol consisted of simultaneous co-contractions of the quadriceps and hamstring muscles either by NMES or voluntary exercise 5 days per week, over a 3 week period. This high intensity NMES treatment was administered for a total of 56.25 minutes («on time» of actual stimulus being delivered to the selected muscles). The investigators of this study reported that NMES was more efficacious than voluntary exercise in minimizing postoperative strength deficits [134].

Snyder-Mackler et al. [219] proposed the use of both high intensity and low intensity NMES protocols for the purposes of making a comparison between the two protocols in their effectiveness in minimizing postoperative strength deficits of the QFM over a 4 week period. The high intensity NMES protocol generated a contraction equivalent to 50% of the uninvolved MVC, and was administered during 3 sessions per week, for a total treatment «on time» of 30 minutes. The low intensity NMES protocol delivered a contraction equivalent to less than 10% of the uninvolved MVC, 5 sessions per week for a total treatment «on time» of 18.7 hours . They concluded that high intensity NMES provided a highly beneficial treatment versus the low intensity protocol, which exhibited no effect during the early postoperative phases of rehabilitation following ACL surgery [219].

Another study by Snyder-Mackler et al. [136] examined the effectiveness of high intensity NMES versus low intensity NMES, in comparison to high volitional exercise over a 4 week postoperative period. Both high intensity NMES and volitional exercise protocols were administered 3 times per week. The low intensity NMES protocol was administered 5 times per week for a total treatment «on time» of 4.5 hours versus 33 minutes of for the high intensity NMES protocol. The investigators

concluded once again that high intensity NMES was highly efficacious in minimizing the postoperative strength deficits of the QFM and that there was no advantage to using a low intensity NMES protocol for these purposes [136].

Finally, Lieber et al. [218] proposed the use of a high intensity type NMES protocol versus volitional exercise in hopes of providing further benefits to patients following reconstruction of their ACL. This NMES protocol consisted of 5 sessions per week, for a total treatment «on time» of 3.3 hours, over a 4 week period. They concluded that NMES provided no further benefit to these patients than volitional exercise and that identical strength gains were observed following treatment with NMES or the voluntary exercise regimen, where treatment activity was matched between groups [218].

Differences in the doses of stimulation among patient samples might account for the discrepancies between our findings (no effect) and those of Delitto et al. [134], Snyder-Mackler et al. [136, 219] and Lieber et al. [218]. In our study, patients in the experimental group received TES for a total of 210 hours «on time» over the 12 week treatment period. It is disappointing to note that this low intensity stimulation protocol (Appendix VI), when used over a long duration, demonstrated only few effects during the early postoperative phase of recovery, following ACL reconstruction.

In regards to our protocol, perhaps the production of a visible muscle contraction would have been more efficacious for the patients in the experimental group. According to Snyder-Mackler et al. [219], there appears to be a threshold contraction intensity of 10% the MVC force of the uninvolved QFM that is required to evoke a training effect that increases muscle function performance. Our TES protocol failed to generate average training contractions with intensities greater than 10% uninvolved MVC in

the involved QFM of these patients. Therefore, this may provide evidence for the lack of treatment effect in our experimental group.

What are the limitations and delimitations of this study?

Although we controlled certain variables during the study, for example, the surgeon, the surgical technique performed, and the rehabilitation protocol being the same for all patients, certain limitations and delimitations prevailed.

Limitations

Number of Recruited Subjects

The number of subjects that we were able to recruit, satisfying the necessary criteria, was limited. As a result, the sample size in this investigation may not have been sufficient enough to detect small treatment effects in the experimental group. Although we had originally proposed a sample size of 30 patients, it took nearly three years to achieve a sample size of 24 patients. Perhaps the recruitment of an additional surgeon performing the same surgical technique would have expedited the process.

Possible inadequacy of Outcome Measurements

It may have been that the outcome measurements were inappropriate for detecting changes in the VM muscle. Because the VM muscle was the primary target of the stimulus protocol, it would seem reasonable to assume that isokinetic force measurements of the overall QFM would mask any functional changes in the VM. Furthermore, during the first postoperative 6 weeks, patients train their QFMs isometrically and perhaps evaluation of strength and iEMG activity would have been more appropriate during the performance of a maximal isometric contraction, due to the specificity of exercise training [213, 220, 221].

Stimulation Protocol

The TES protocol required a minimum of 6 hours of stimulation per night, 5 nights per week. This parameter was changed from the original paradigm, in order to minimize the lack of compliance due the overall greater demands of this protocol, which stated 8 hours minimum per night, 6 nights per week. Furthermore, with regards to compliance, the built-in meter used to track patient compliance was faulty 90% of the time. Without this feature, we were unable to track the actual patient usage and therefore, were unable to confirm patient compliance as stated on their individual log sheets.

Delimitations

The delimitations of this study include the recruitment of volunteers, the injury itself, degree of preoperative QFM weakness and atrophy, the timing of surgery and the trauma of the graft harvesting procedure.

Volunteer vs. Non-Volunteers

In our study, the recruitment of subjects was on a volunteer basis and volunteers, in turn, are often better motivated. It is common knowledge that motivation, self-perceived capabilities, degree of compliance, and the belief in the efficacy of treatment can influence the physical performance or therapeutic outcome. Therefore, these motivational factors would likely influence the results of our investigation.

Timing of Surgery

How the initial injury to the ACL took place and the interval between the original injury and ACL reconstruction, was greatly variable among the different patients. It is common practice to undergo physiotherapy for a period of 6 months to 1 year prior before recommending invasive surgery to replace a ruptured ACL with a brand new reconstructed ligament (Dr. E Lenczner, personal communication). During this time, patients are advised to wear a protective knee brace both at the early stages post-injury, and during recreational sporting activities. Furthermore, depending on the course of action undertaken post injury and at the time surgery, atrophy and subsequent weakness of the QFM will vary to different degrees.

Future studies on postoperative ACL patients should take this into account when making comparisons between different treatment groups and postoperative rehabilitation programs. To circumvent this variability, perhaps a better approach would entail recruitment of patients with a fixed time delay between injury to surgical intervention. According to Shelbourne et al. [208], patients who delayed surgery by 2 months, demonstrated faster progress in postoperative QFM strength when compared with patients who had surgery only 11 days following injury. Therefore, in light of this observation, a minimum delay of 2 months should be required when deciding on a fixed time delay.

Graft Procedure

The patellar tendon autograft is commonly referred to as the gold standard for ACL reconstruction. The benefits of the medial third bone-patellar tendon-bone (BPTB) graft include its high tensile strength and fixation

with bone to bone healing [222]. Reported drawbacks of the patellar tendon autograft include patellofemoral pain (PFP), patellar tendinitis, patellar fracture and impairment of the QFM mechanism, as a result of the graft harvesting procedure [223]. PFP imposes limitations on complete extension of the knee joint which, in turn, inhibits proper activation of the QFM. This would make complete rehabilitation of the QFM difficult and delay the progress in strength recovery during the early postoperative period [224-226]. Patients with PFP would introduce a confounding variable into the study and possibly skew the results of the ensuing investigation.

Alternative graft choices, such as the semitendinosus and gracilis autografts, have generated similar results to the BPTB autograft, in terms of postoperative stability in the early stages of recovery [222]. However, unlike the BPTB autograft, the harvesting procedure relative to the semitendinosus and gracilis autografts, spares the trauma to the QFM mechanism [222]. Therefore, we recommend the use of and alternative autograft procedure in order to avoid the morbidity reportedly caused by the harvest of the patellar tendon autoigraft, when studying ACL patients during the early postoperative stages.

Conclusions

In summary, improvement of function, activity level and muscle strength in the QFM was evident in both the experimental and control groups, however, no significant differences were found between the two groups, during the early postoperative stages of recovery following ACL reconstruction. Therefore, it was concluded that TES, in addition to

rehabilitation, has minimal extra benefit for patients who have undergone ACL reconstruction.

APPENDIX I

Anterior Cruciate Ligament Surgical Reconstruction Procedure

- mild line incision

- muscle biopsy distal vastus medialis

- removal 10mm strip of central one third pattella tendon - full thickness - with bone blocks from distal patella and proximal tibial tubercle

- arthroscopic joint debridement and meniscal surgery if needed

- arthroscopic notch plasty

- drilling of tibia 10mm tunnel and femur 10mm tunnel

- insertion of patella tendon autograft in isometric position

- fixation of bone plugs in femur and tibia with interference screws

- wound closure, closing patella tendon sheath, bone graft patella

APPENDIX II

Patient Consent Form

HUMAN RESEARCH CONSENT FORM

TITLE: A Study of the Efficacy of Therapeutic Electrical Stimulation in Minimizing Disuse Muscle Atrophy and Dysfunction of the Quadriceps Femoris Muscle Following Knee Surgery: A Model for Microgravity-Induced Disuse Atrophy.

SPONSORS: This study will be conducted at the Montreal General Hospital as a joint project between University of Montreal and the Canadian Space Agency

TYPE OF STUDY: Postoperative study on orthopaedic patients following reconstruction of their anterior cruciate ligament.

INVESTIGATORS:

Phillip Gardiner PhD
Professor Department of Physical Education
Université de Montréal

Karen Pape MD FRCP(C)
Medical Director Magee Clinic

Christina Hui-Chan Ph.D
School of Physical and Occupational Therapy
McGill University

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David R. Williams MSc MD CM CCFP FRCP(C)
Astronaut, Canadian Space Agency,
Assistant Professor of Surgery, McGill University

Carrie Olha BSc
Graduate Student, University of Montreal
Research Assistant, Canadian Space Agency

PURPOSE AND DESIGN OF THE STUDY

This is a research study designed to evaluate the effects of neuromuscular electrical stimulation (NMES) on preventing disuse muscle atrophy (muscle shrinkage related to inactivity) and inadequate muscle function in postoperative orthopaedic patients. Significant atrophy and weakness of the quadriceps femoris muscle (QFM) or thigh muscle group occurs following knee injury and anterior cruciate ligament (ACL) reconstruction. The ACL is a ligament of the knee just underneath the knee cap which helps to attach the thigh bone to the shin bone. The purpose of this study is to conduct preliminary experiments to determine if the stimulation pattern is effective in minimizing muscular wasting that arises in the lower extremities of astronauts following prolonged spaceflight. Using ACL reconstructed patients, provides a good

following prolonged spaceflight. Using ACL reconstructed patients, provides a good model for studying treatments that can minimize such deficits in muscular function. The treatment of NMES will be administered in conjunction with a standardized rehabilitation program.

Prior to surgery I will come to the lab at the University of Montreal (U of M) for an orientation session and I will be assigned to one of two groups which will differ in the procedures to be undertaken during my recovery. **Both groups are required to follow the standard rehabilitation program as outline by Dr. Lenczner in appendix 3.** If I am assigned to **group (1) control, no stimulation** is required and I will **only** be required to follow the standard rehabilitation program. If I am assigned to **group (2) experimental group**, I will be required to follow a nightly stimulation protocol consisting of **low intensity muscle stimulation** in addition to the rehabilitation. If I am assigned to the experimental group, I will be instructed in the proper use of the electrical stimulation unit and the associated protocol along with the goals of the stimulation program.

After the orientation session, I will be asked to perform a series of leg contractions to evaluate the strength of the quadriceps femoris muscle groups in both legs. These contractions will be evaluated on a machine that measures muscle strength called an isokinetic dynamometer. Surface electrodes (rubber patches placed over the skin) will be placed over this muscle group to record the activity in the muscle as I perform 2 sets of 3 repetitive contractions. At the time of contraction, if I experience pain, I will be asked to rate my pain on a graphic rating scale (Appendix 1). I will also be asked to undergo measurements of my legs and magnetic resonance imaging (MRI) will be taken prior to my surgery.

The MRI apparatus is a large hollow cylindrical drum that is similar to an x-ray but the image obtained does not require the use of radiation. I will be asked to undergo an MRI whereby I will be lying horizontally and totally submerged, from head to toe, inside this cylindrical drum. I will be asked to lie still for approximately 1 hour as the machine proceeds to take pictures of my legs. As the machine is operating, I will hear a series of knocking sounds, like someone using a hammer and tapping on the outside, but this should not concern me since this is how the machine normally operates to take the pictures needed. Finally, I will be asked to allow the surgeon to perform a muscle biopsy (small tissue sample) during surgery to provide one sample of muscle tissue (approximately half the size of a corn kernel) from my involved quadriceps

Following surgery, I will remain in the hospital for a minimum of 24 hours and follow the recovery procedures advised by the orthopedic surgeon as outline on page 1 of appendix 3. Depending upon which group I will be assigned to, I may receive low intensity electrical stimulation applied to the surface of the extensor muscles (QFM) of my involved knee. This type of stimulation will be a mild tingling sensation administered for 6-8 consecutive hours while I sleep. A pair of electrodes will be applied over the vastus medialis and rectus femoris muscles of my involved limb. The positions of these electrodes will be marked using a non-toxic water-soluble marker so that I will be able to reproducibly apply them to the correct positions at home. Once familiar, I will begin the treatment for the selected duration and record my subjective responses to the electrical stimulation as outline in the "Patients Stimulation Log" (Appendix 2). If I find the stimuli uncomfortable or disruptive to my sleep, I will be able

to inform my nurse, or a member of the research team. who will adjust the stimulation to more comfortable levels.

GROUPS 1 and 2

The next day, following the advice of the orthopaedic surgeon, I will be discharged from the hospital and will begin a standardized physical therapy program (Appendix 3) for a period of 12 weeks.

GROUP 2

For the next 12 weeks, in combination with physical therapy, at **home** I will be required to wear and activate the stimulation unit according to the protocol. I will place the electrode patches over the marked positions and each day I will keep a record of my stimulation schedule and record my subjective feelings and any problems I may have on the "Patients Stimulation Log" I've been provided with. **I will be allowed to take 2 rest days each week whereby I won't have to stimulate my leg but these days must not be consecutive.**

Within two weeks from the day of surgery, I will be asked to return to U of M for a follow-up visit with the supervising orthopaedic surgeon for approximately 30 minutes. At this time, I will be asked to bring my log sheet and the stimulator, and proper electrode placement will be verified.

GROUPS 1 and 2

At 6 weeks following my surgery (postoperatively), I will be asked to return to U of M for approx. 2 hours with my log sheet to repeat the dynamic leg contractions in order to evaluate the recovery of my muscle strength in the involved limb. Pain measurements will also be recorded as before.

At 12 weeks postoperatively, I will be asked to return to U of M for approx. 2 hours to repeat the testing procedure previously undergone in the preoperative session. These will include the dynamic leg contractions, pain assessment and another MRI will be scheduled. Following the testing procedures, I will be asked to undergo a muscle biopsy to provide another sample of muscle tissue (approx. 1/2 the size of a corn kernel) from my involved leg. The study will then be terminated and I will be asked to return all the equipment and the completed log sheet.

If at the end of the study, the data collected indicates that the stimulation protocol helps muscle recovery, then if I am assigned to the control group, I will be given the opportunity to receive stimulation at the end of the study.

POSSIBLE BENEFITS OF PARTICIPATION IN THE STUDY

The potential benefits of my participation in this study, although not assured **(there is the possibility of no benefit)**, include:

1) Reduced strength loses and reduced pain in the early postoperative phase.

2) Augmented gains in range of motion around the knee joint.

3) Increased rate of return to full athletic activity.

Clinic-based stimulation protocols, which often can be uncomfortable, require the presence of an experienced technician and therefore, the patient must travel to a facility to receive treatments. This may not be achievable over an extended period of time since the patient must make time during working hours to undergo the stimulation. In alternative to clinic-based stimulation , these home-based stimulation protocols are easy to use, with minimal discomfort to the patient. Furthermore, home-based stimulation would be more practical for the patient and if proven to be effective, it may allow for less time to be spent in a supervised rehabilitation program. Thus, the patient would be allowed to stimulate in the comfort of his/her home, accelerate the overall rate of recovery and resume full activity more rapidly with less risk of injury to the knee or graft.

The result of this study will contribute to the assessment of NMES in minimizing or preventing the physiological adaptations that arise from periods of disuse and unloading. These include knee ligament injury, cast immobilization, bedrest following surgery and periods of weightlessness during prolonged spaceflight. While the global objective of this proposed study is to evaluate the potential efficacy of new medical technologies in preventing some of the medical problems associated with human spaceflight, the data will have significant implications in the field of rehabilitation medicine.

FORESEEABLE INCONVENIENCES, DISCOMFORTS AND RISKS

Surgical reconstruction of the injured ACL is performed on the basis that persons with an ACL deficient knee, are prone to further joint injury. The ultimate goal is to prevent further injury and retard the onset of degenerative arthritis, while allowing the person to continue to participate in demanding athletic activities. Profound atrophy and weakness of the quadriceps femoris muscle, related to inactivity (disuse), can occur as early as the first few days following knee ligament injury and ACL reconstruction. NMES has been used as a treatment to minimize muscular atrophy and weakness related to many musculoskeletal and neuromuscular disorders. It has also been used on ACL patients as a rehabilitative tool to attenuate postoperative disuse atrophy and pain. While the interventions described above are standard clinical procedures, they still may involve possible risks.

A)   I may experience some discomfort due to the electrical stimulation and the wearing of skin surface electrodes, such as minor skin irritation.

B)   I may feel some discomfort (slight sting) during the injection of the local anaesthetic prior to insertion of muscle biopsy needle, as well as additional soreness a day or so after at the insertion site. Infection is possible but unlikely due to the sterile conditions used.

C)   The potential risks associated with muscle biopsies include:
1. Allergic reactions to local anaesthetics
2. Post-biopsy infections

3. Damage to underlying anatomical structures
4. Excessive bleeding
The incidences of these however, are extremely rare (< 1 in 1000)
If any of these should arise immediately contact Dr. Eric Lenczner at 989-1231

D)   I may experience muscular soreness or injury following the testing procedure or upon return to exercise following the study period so care must be taken to exercise progressively.

E)   I may experience anxiety or claustrophobia during the MRI analysis procedure however, MR imagery does not produce any known health risks or rely upon the use of intravenous chemicals. Therefore, for these reasons, no potential risks associated with an MRI are foreseen.

Measures taken to minimize these risks will include:

1. Close supervision by an individual specially trained in the use of isokinetic dynamometers.
2.   Only skilled individuals specially trained in the percutaneous needle biopsy technique will be employed.

EMERGENCY TELEPHONE NUMBERS

1) Dr. Phillip Gardiner: (514) 343-7450
2) Dr. N.B. Whittermore: (514) 934-8087
3) Dr. E. Lenczner: (514) 989-1231

INQUIRIES CONCERNING THE STUDY

I understand that all the questions I may have about this study will be answered by Miss **Carrie Olha** and/or her supervisor **Dr. Phillip Gardiner** at the Centre d'Education Physique et des Sports (CEPSUM), Université of Montréal, 2100 boul. Edouard-Montpetit Room 8205, Montreal, Québec, (514) 343-7450. If I have any questions concerning my rights as a study participant or in the event of a research related injury, I can contact **Dr. N.B. Whittermore**, the Director of Professional Services at the Montreal General Hospital at 934-8087.

In order to monitor compliance with institutional regulations regarding research involving human subjects, the Research and Clinical Trials Committees reserve the right to review the research records at the MGH and MGH Research Institute.

WITHDRAWAL FROM THE STUDY

I understand that my participation in this research study is strictly voluntary of my own volition. I may refuse to participate or withdraw from the study at any time which will not involve any prejudice or penalty to the health care or the benefits to which I am entitled. I understand that my participation in this study may also be terminated with or without my consent and that the study has been approved by the Ethics Committee of the Montreal General Hospital. In addition, any personal information obtained from me through this study will remain confidential and participants will not be identified in any communication concerning this work. This confidentiality will be maintained whereby each file will be assigned a code and this code will only have significance to the investigators in this study. Furthermore, the coded files will be kept in a locked filing cabinet to which only the investigators will have access.Lastly, I will be given a copy of this consent form for my future references.

PARTICIPANTS DECLARATION

I,_____agree to participate as a subject in a
Printed Name of the Subject
research study designed to evaluate the effects of two modalities of neuromuscular electrical stimulation (NMES) on preventing disuse muscle atrophy and muscle weakness in the lower extremities.

I have understood all of the testing and experimental procedures described in the preceding form.

I am aware of the possible foreseeable harmful consequences that may result from such participation, and that such participation may otherwise cause me inconvenience and discomfort as described in the preceding form.

I also understand that by signing this form I do not give up any legal rights.

Lastly, I have had the opportunity to ask questions and I have received satisfactory answers to each question I have asked.

Signature of subject:_____ Date:_____

Print Name:_____

DECLARATION OF THE INVESTIGATOR

I, Carrie Olha certify that I have fully explained the above-mentioned subject matter, the nature of the experiment, the known risks involved in participating in this study, and the fact that he/she has the right to withdraw from the study at any time, without prejudice.

Signature of researcher:_____ Date:_____

Signature of witness:_____ Date:_____

Print Name:_____
(witness)

APPENDIX III

Rehabilitation Protocol following ACL reconstruction

ACL PROTOCOL - Dr. E. Lenczner.

Prepared in conjunction with S.Cross pht., C.A.T. (C). and the McGill University Sport Medicine Clinic. Revised April 1995.

PRE-OPERATIVE PERIOD

PROTECT the torn ligament(s) and/or meniscus during the treatment
Increase the ROM (bike, AROM, etc.)
Decrease the swelling/effusion as much as possible
Maximize the strength pre-op
Quads, hamstring, calf flexibility
Good proprioception
Normal gait pattern
Avoid cutting activities, stick to straight ahead exercises/sports (treadmill, cycling)

- Educate the patient about the stages of the rehabilitation post-op especially the immediate period around the surgery. Review the necessity of icing frequently.
- Make sure the patient can use crutches pre-op.
- Make sure the patient is familiar with isometric Quads, Hamstrings, Gluts as well as ankle pumping/ROM exercises.

POST-OPERATIVE PERIOD
0-2 weeks

Check for DVT
Check for abnormal pain or abnormal swelling
Change dressing if necessary, observe healing status of incision
Feather WB-PWB (crutches)
Gentle weight shifting side to side with the splint
Wear Zimmer splint when not icing or working ROM
Wear ace wrap for compression to control effusion and swelling
AAROM-AROM knee flexion, begin bike for ROM exercises
Maintain extension
AROM ankle pumping
Isometric Quads, Hams, Gluts
Hip ABDuction, ADDuction, SLR and prone SLR with the brace
Hamstring stretching
Patellar Mobilizations
Pool therapy program can begin once incision healed (remove the brace)
Ice the knee after the treatment, and advise the patient to ice at home several times daily
F/U at 1 week post-op.

2-4 weeks

PWB-FWB with Zimmer splint (crutches vs. cane).
Continue ROM for flexion, bike should be progressed
Maintain or increase extension (overpressure or gentle tibiofemoral mobilization)
Continue with isometric Quads, Hams, Gluts, if necessary
Continue with hip SLR, prone SLR, and ABD/ADD
Toe raises for calf strengthening
Hamstring stretching
Calf stretching
Patellar mobilizations
Scar mobilizations, can use Vitamin E gel capsules to massage into the incision
Massage/friction patellar tendon to decrease scar tissue formation
Continue the pool therapy program (if the incision is well healed)
Ice the knee after the treatment

4-6 weeks

Patient should be FWB if the knee is in **<u>full extension</u>**
D/C Zimmer splint
Continue flexion/extension ROM and increase biking
Continue hip exercises and increase resistance
Closed kinetic chain exercises for quads (step ups, etc...)
+++ Hamstring strengthening both concentric and eccentric
Continues toe raises and progress to one foot toe raises
Continue Q+H flexibility
Continue patellar mobs
Continue scar mobs
Balance on one foot to begin proprioception training
Ice the knee after the treatment.

6-8 weeks

Bike for endurance, strength and ROM
General strengthening for the lower extremity with closed chain exercises for the
quads
Biodex/Cybec with **ANTISHEAR BAR** at > 120 degrees per second. (ACL Brace is
<u>NOT</u> good enough support for resisted knee extension).
Continue with Q+H flexibility
Ice the knee after the treatment/exercises

8-12 weeks

Ensure full ROM for flexion/extension
Continue strengthening, progress eccentric quadriceps work in closed kinetic chain
and/or with the antishear bar on the Biodex/Cybex
Jogging in a straight line if isokinetic test is > 75 - 80%
Continue stretching/flexibility
Ensure good patellar mobility
Patellar tendon should be clear of scar tissue/thickening
Continue/progress proprioception exercises
Ice the knee after exercises

12-16 weeks

Strength and endurance work
Jogging (only in a straight line, **no cutting should be done yet**)
LE stretching
Proprioception ++

16-24 weeks

Continue strength and endurance work
Continue flexibility
Increase running, cycling
Begin plyometric drills
Begin to work on **sport specific drills** with gentle cutting and progress as tolerated

At 6 months

Make sure the patellar tendon is clear of scar tissue/swelling
Make sure knee ROM is full
Make sure the patellar mobility is normal
Make sure the flexibility is good
Isokinetic test
Return to sports (may need a longer period depending on the sport)

APPENDIX IV

Differential EMG Electrode Placement over the Vastus Lateralis and Vastus Medialis

Vastus Lateralis

Vastus Medialis

Reference Electrode
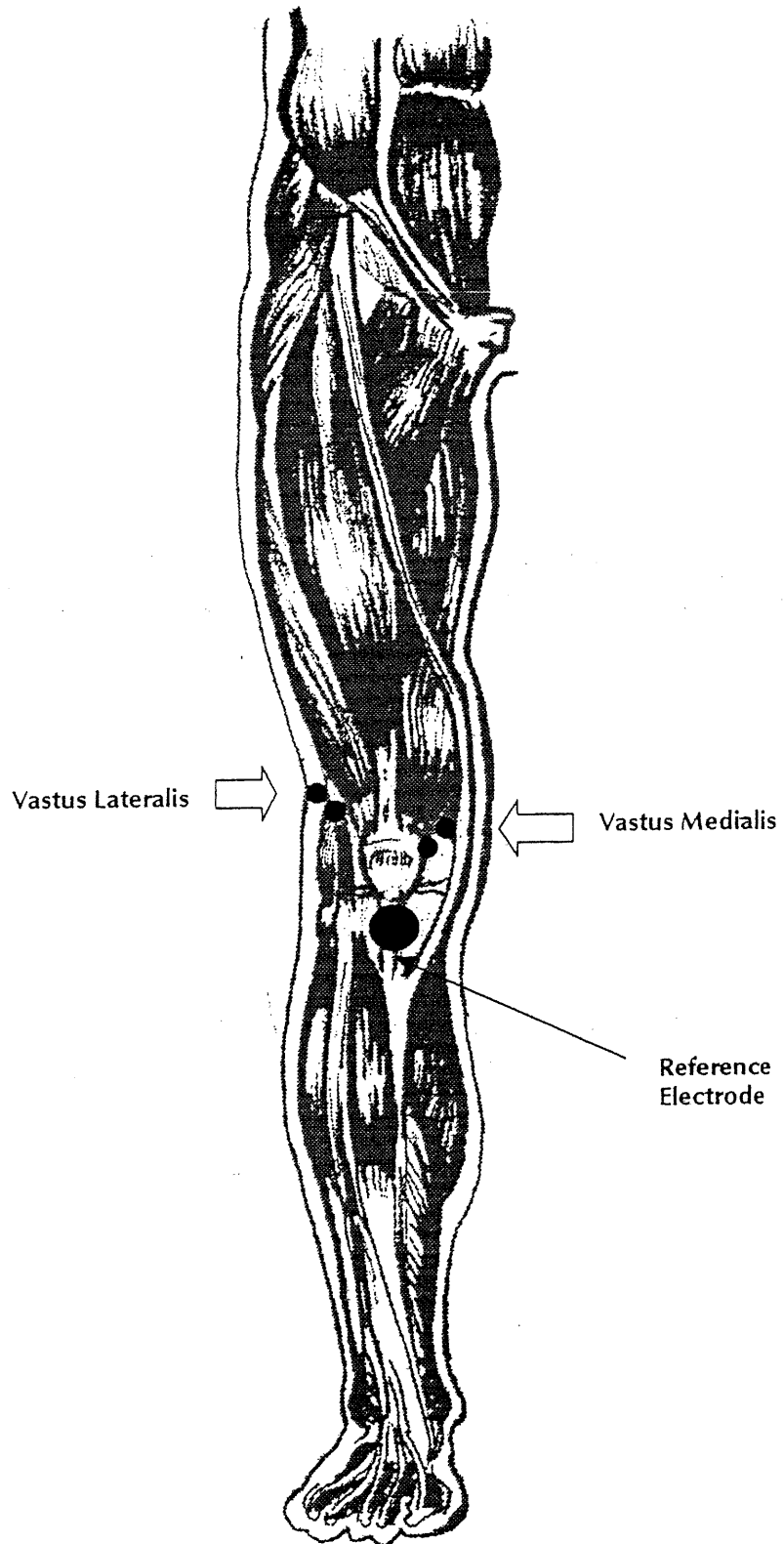
APPENDIX V

Data Acquisition System Instrumentation
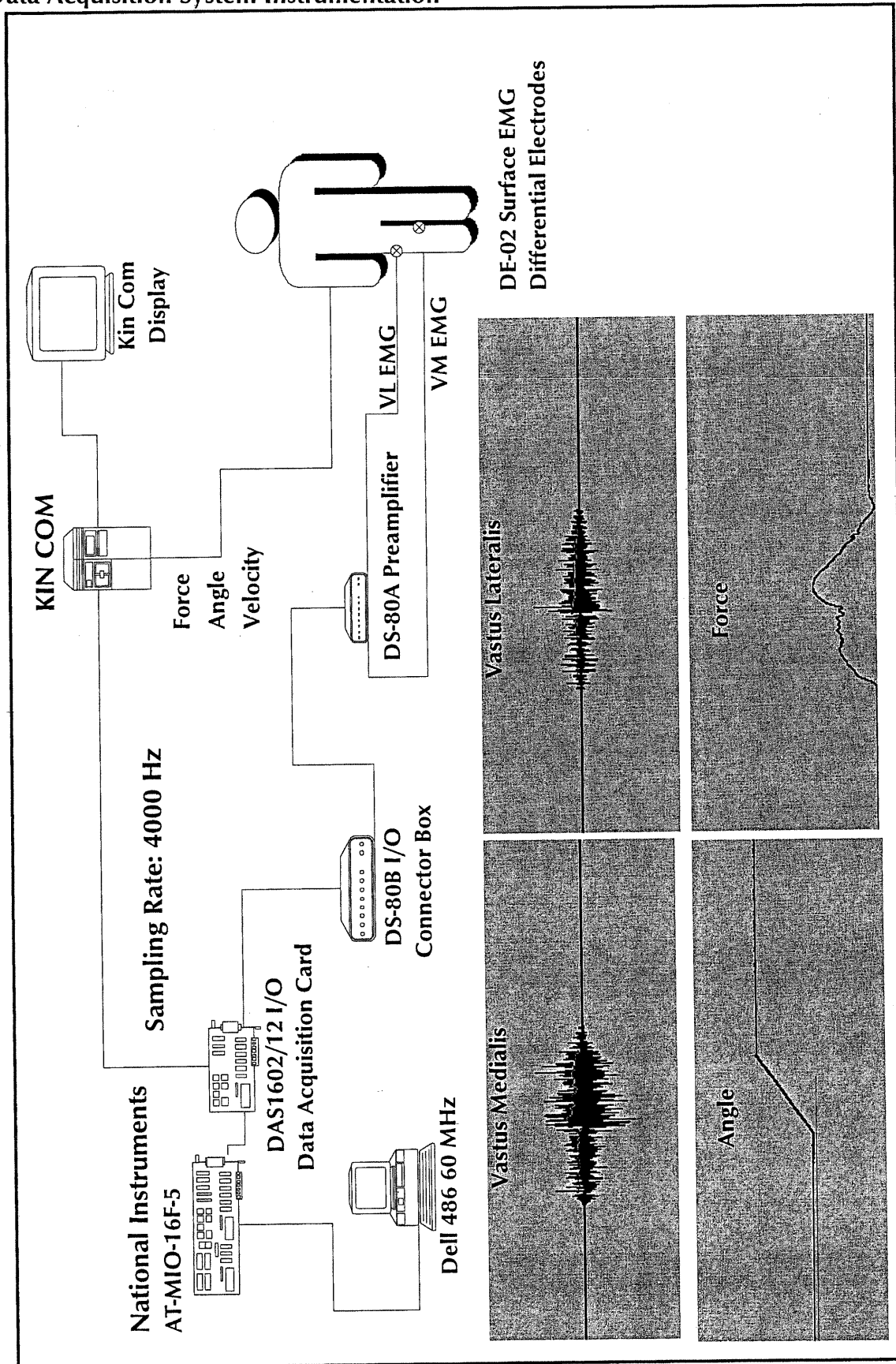
APPENDIX VI

Nocturnal implementation of TES - Stimulation Parameters

       •Waveform: Assymetrical biphasic pulse

       •Pulse Rate: 35 Hz

       •Pulse width: 300 μsec

       •Peak intensity: < 10 mA

       •Ramp time: 2 seconds

       •On time: 12 seconds

       •Off time: 12 seconds

       •Treatment time: Continuous

       •Duration: 6-8 hrs each night for the duration of the study.

QUADRICEPS

GOAL

- Increase hip and knee stability
- Improve ambulatory skills
- Improve endurance

MUSCLES

- Vastus Medialis
- Vastus Lateralis
- Vastus Intermedius
- Rectus Femoris
- Sartorius

FUNCTION

- Knee Extension
- Hip Flexion

Sartorius

Rectus Femoris

Vastus Lateralis

Vastus Medialis

QUADRICEPS

TO ISOLATE THE QUADRICEPS :

- expose the leg from the groin to the knee
- view from above with the knee flexed in a seated position
- resist knee extension to define rectus femoris

- the quadriceps narrow and become a tendon a variable number of inches above its patellar insertion
- landmarks include the patella, patellar tendon and the inguinal crease
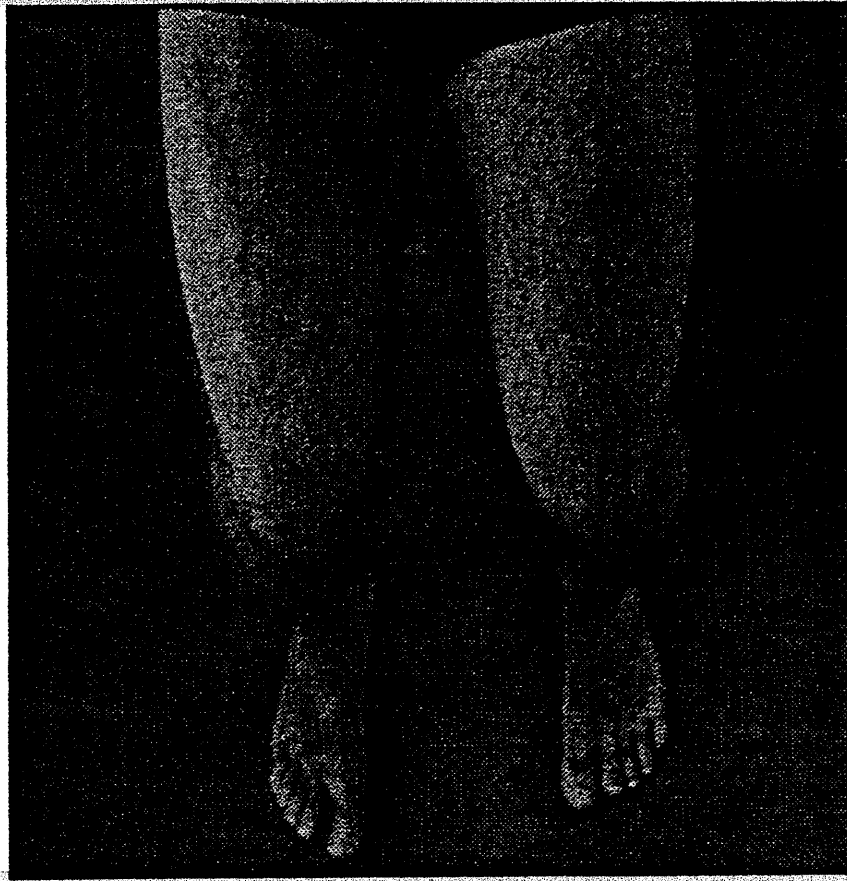
QUADRICEPS

BLACK ELECTRODE :

- Lower (black) electrode should be placed first
- place on the belly of the vastus medialis muscle
- Draw an imaginary line from the inner edge of the patella and place just outside to this line
- Approximately 5 to 7 cm from the superior patella border.

RED ELECTRODE :

- Upper (red) electrode should be placed centrally over the belly of the rectus femoris muscle
- Place approximately two thirds of its length, as measured from the superior patella border
- Should be parallel to the lower (black) electrode
- Electrodes should not be more than 15 cm apart ; if they are too far apart, the treatment will be less effective
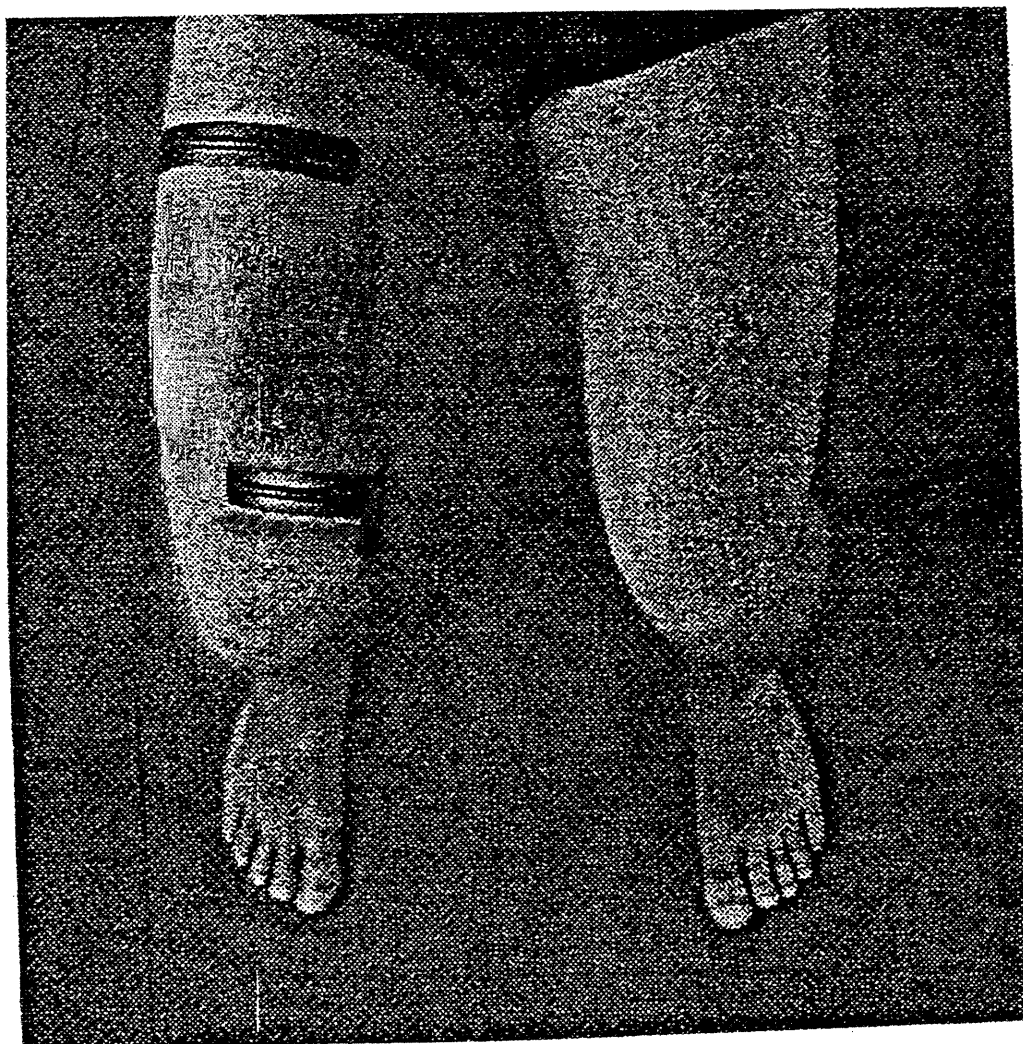
TRAINING MANUAL

Guidelines On The Proper Use And Maintenance Of The ONE 2 ONE Stimulator

Revised and Printed with Permission from Mayatek Inc.

OVERVIEW

Neuromuscular electrical stimulation (NMES) is part of the core curriculum in most schools of Physical Therapy. This electrical stimulation of muscle is recognized therapy widely used in sports training and orthopedic rehabilitation.

CAUTIONS

You will be asked to sign a consent form indicating that you have been made aware of a few major safety precautions. They are repeated here for your reference. **Federal law in the USA restricts this device to sale by or on the order of a physician. A similar restriction is advised in Canada.**

INDICATIONS

The Food and Drug Agency (FDA) in the United States has accepted Electrical Stimulation for the following uses:

- for the prevention or retardation of disuse atrophy;
- for maintaining or increasing the range of motion;
- for muscle reeducation;
- for relaxation of muscle spasms;
- to increase local blood flow.

CONTRA-INDICATIONS AND WARNINGS

As the effects of NMES during pregnancy are unknown, it is recommended that stimulation not be used while you are pregnant or if you are planning to become pregnant.

The electrical current of the stimulator may effect patients with known myocardial arrythmias, or the output of a pacemaker. The stimulator must not be used by, or demonstrated on anyone with, a pacemaker or patients with known myocardial arrythmias.

Stimulation should not be used on cancer patients or on patients with suspected or diagnosed epilepsy.

You should not stimulate over swollen, infected or inflamed areas or skin eruptions such as phlebitis.

This device should be used only under the continued supervision of a trained professional for the treatment for which it is prescribed. The device is designed for external use only.

PRECAUTIONS AND ADVERSE EFFECTS

Occasionally, skin irritation may occur at the site of the electrode placement following long-term application. Sometimes, electrode burns may occur at the electrode site. Please ensure that the stimulator power is turned off before removing or applying electrodes.

GETTING TO KNOW THE EQUIPMENT

THE STIMULATOR

The ONE 2 ONE neuromuscular electrical stimulator is a small rectangular box with a display window and a control panel protected by a sliding front cover. To access the controls, slide the front cover down. The power switch is located on the left side of the face of the stimulator just below the display window.

The ONE 2 ONE stimulator has two separate channels allowing two different muscle groups to be stimulated at the same time through different sets of electrodes. **However, for the purposes of this study, only Channel 1 will be used.**

THE WIRES

The ONE 2 ONE will be supplied with one lead wire. This lead wire has a black connector with two prongs at one end and a split plug (one red and one black) at the other end. This lead wire consists of two wires joined together throughout most of its length, but which splits into two at the plug end. They can be split as far as necessary to accommodate the electrode placement pattern designed for you.
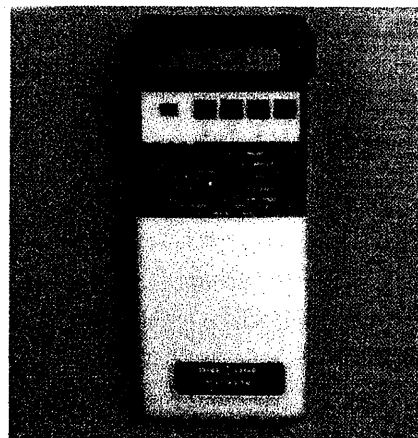
ELECTRODE PATCHES

The ONE 2 ONE will be used with one type of electrode patches which are latex-free. Complete instructions for their application and use will be provided by the investigators of this study. You will be provided with two complete sets of patches to start your program. The size of the electrodes to be used will vary depending on the individual.

THE CLIP ATTACHMENT

The clip on the back of the unit can be used to secure it so it won't move around during the treatment. If the clip is not needed, use a screwdriver to remove it.
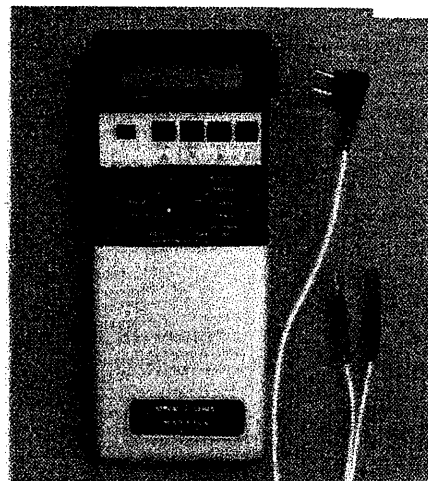
THE BATTERY

The ONE 2 ONE is powered by a 9-volt battery. Disposable 9-volt (alkaline) batteries will be provided at each visit during the study. Storing extra batteries in the refrigerator will promote durability.

INSERTING THE BATTERY

1. Be sure the power is in the "OFF" position. Remove the cover of the unit by sliding it completely off to expose the battery compartment.
2. Lift the battery connector plate by placing a finger underneath it and holding it in the raised position.
3. Snap the new 9-volt battery into the connector.
4. Slide the cover into the grooves on the side of the case and push it up until the battery and control panel are concealed.

When you turn the unit on, if the amber light stays on rather than flashing once, the battery must be replaced.
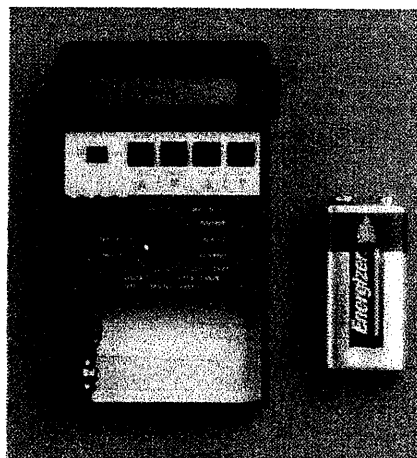
REPLACING THE BATTERY

The battery should be replaced every **7 days** to avoid any complications with the overall treatment time. The new battery should be placed into the unit within **3 minutes** of removing the old one. This will keep the built-in memory of the unit intact.

1. Be sure the power switch is in the "OFF" position and remove the front cover of the unit.
2. Tip the stimulator so that the battery turns outward. Place a finger underneath the battery connector plate to hold it in position. Then carefully remove the old battery from the connector.
3. Snap the new 9-volt battery into the connector. Slide the cover into the grooves on the side of the case and close it.

NOTE: This unit must never be plugged into an electrical outlet. Never use 9-volt adapters.
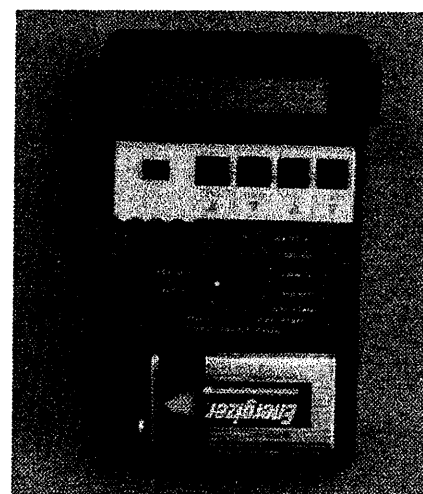
LIGHT SEQUENCE

The red light indications are as follows:
- Flashing 1 time per second: No current flow
- Flashing 8 times per second: Current ramping up to stimulation level
- Continuous red light: Current is flowing
- Flashing 4 times per second: Current ramping down from stimulation level

HOW TO OPERATE THE STIMULATOR

GENERAL INSTRUCTIONS

Plug the black two-pronged wire connector into the output jack on the left side (channel 1) of the stimulator. The wire must be inserted so that it hangs down at the side of the stimulator as seen in the photo. **Never attempt to plug a connector into an electrical outlet.**

Leaving the wire connected to the stimulator between uses reduces wear. It may be taped across the back of the unit as illustrated.

To prepare the electrode patches, refer to the instructions on the package. The red wire lead plugs into one electrode and the black wire lead into the other electrode. Connect the wires by inserting the pin into the hole in each electrode. Push the pin in all the way until no metal is left exposed.

TREATMENT TIME

The stimulator should be used for a minimum of 8 hours, five nights per week. You will be allowed to take two **non-consecutive** nights off per week. As body growth and repair take place while you sleep, this program is to be used only at night. Together with the investigator of this study, you will determine the level of electrical current that is comfortable for you. You should be able to feel a slight sensation around the electrode area, whenever the lights on the control panel indicate that the current is on. This slight sensation has been described as a "tickle". You should not feel muscles contracting or toes responding to the current. You should not find that the stimulation is affecting your ability to fall asleep. It may take a few nights to get used to the stimulation while sleeping. If you find that your muscles ache in the morning after a night of therapy, the setting is too high. Please call the investigator to discuss this problem.

Do not be surprised if you do not feel the stimulator when you wake up in the morning. Some people habituate to the stimulation sensation and become unaware of it after a time. This does not mean it is no longer working. It just means that you have "blocked out" the awareness of the stimulation (similar to blocking out loud street noise within weeks of moving to a new home). It is not a signal for you to turn the stimulator up.

BEGINNING STIMULATION

Once you have connected the lead wire to both the ONE 2 ONE stimulator and to the electrodes, place the electrodes on the leg according to the chart provided. Before turning on the power, verify that the program switch is in the correct position (NT 1). Align the arrowhead of the switch knob to **NT 1** on the front panel.

You are now ready to turn the unit on. Turn switch to the "ON" position. A short delay will occur while the system is initializing. The red light will begin its cycle. The yellow light on the right hand side should blink once and go off. If it stays on, replace the battery.

You must set the amplitude of Channel 1 each night. When you turn the machine on, a "1" appears in the display and the word "AMP" appears in the upper left corner. You are now ready to adjust the amplitude of the stimulation for Channel 1.
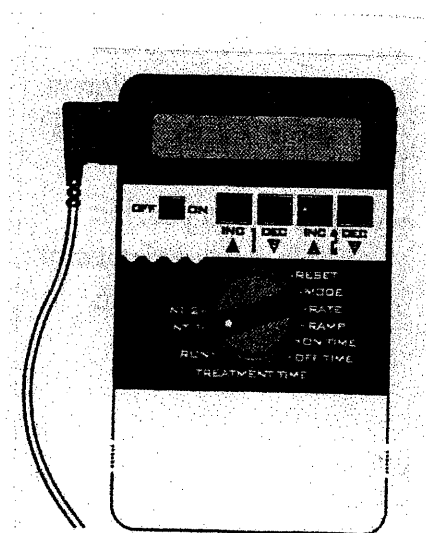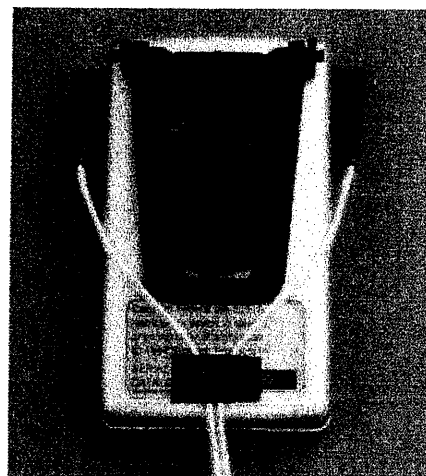
To increase the amplitude, press the button marked INC 1. To decrease it, press the button marked DEC 1. Adjust it slowly until you obtain the required setting.

Your setting is :_____

Always exercise caution when increasing the amplitude setting. It is recommended that you increase the amplitude one milliamp at a time by repeating single button pushes. The increase will be present after the two second ramp up. Holding the button down causes the amplitude to increase quickly to the maximum output. **You should never do this since a painful muscle contraction will result and you could damage the muscle being stimulated.**

Now slide the front cover to its closed position. This will prevent the settings from being moved to an incorrect level during the night.

As a safety feature, NT 1 programs have been designed with a maximum output of 25 milliamps. This level of current will cause a contraction strong enough to waken you, but would be unlikely to cause significant muscle damage in the short term.

CHANNEL 1
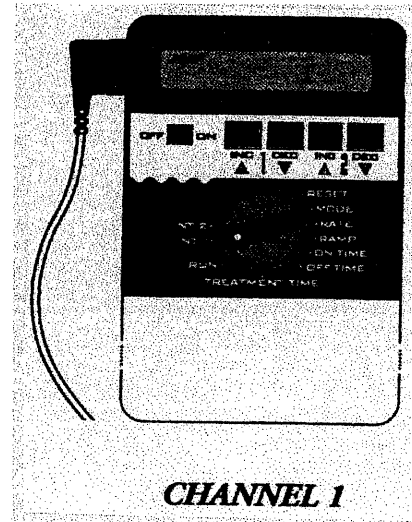
TREATMENT TIME RECORD
Sixty seconds after turning the stimulator on, it will begin to record the number of treatment minutes. A flashing number will replace the intensity and "AMP" displays. This number increases each minute to provide a visual record of treatment time. When the machine is turned off in the morning, it will reset automatically for the next night.

SAFETY SHUT-OFF
If the wires or electrodes come loose from your leg, the ONE 2 ONE stimulator will automatically shut off.

Should this occur, the red light will remain on and the readout will indicate zero. Turn the on/off switch to the "OFF" position and reconnect the wires and/or electrodes. Once the electrodes and wires are refastened, turn the stimulator back on and reset the machine to the prescribed level.

If you turn up the stimulator intensity without connecting the wires and electrodes to the stimulator and the leg, the display will read zero.

IN THE MORNING
To keep track of the number of minutes of treatment received overnight, record the flashing number on the display screen onto your log sheet prior to turning the stimulator off.

Always turn the stimulator off before attempting to remove the lead wire or electrodes or you may receive a shock.

To turn the stimulator off, open the front cover and turn the power switch to the "OFF" position. Remove the electrodes and store according to the instructions on the package.
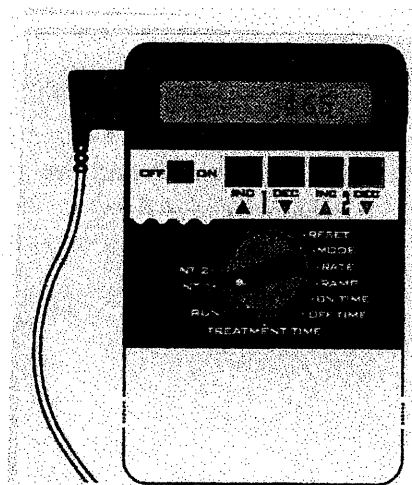
NOTES ON POTENTIAL ADVERSE EFFECTS
Skin irritation may occur in the area covered by the electrodes. If they are not placed accurately, there is a slight risk that the wrong muscles will be stimulated. If the stimulator is set too high, there is the possibility -f - large muscle contraction which can cause pain in the muscle and could result in damage to tendon or graf xxii

Do not exceed the maximum recommended setting or change electrode positions on your own.

Your NMES program may be continued during the common cold. You should not use the stimulator during significant illness, especially if you have a fever. Take a break from the therapy until you have recovered, then begin again. **Please keep track of and report any prolonged periods off NMES.**

Discuss with the investigator if one of these situations develops:
- if you are experiencing any pain,
- if a rash or skin irritation occurs at the site of the electrode,
- if the machine does not seem to be working properly, or
- if you have any questions or problems, no matter how silly they may seem.

CLEANING THE EQUIPMENT

The only maintenance necessary on your ONE 2 ONE stimulator is occasional cleaning and battery care. Wipe the unit gently with a soft cloth or sponge dampened with water to remove dust and dirt.

Rubbing alcohol may be used to remove stains or adhesives that stick to the case. Do not use strong household cleaners as they may damage the plastic parts. Never immerse your ONE 2 ONE in liquid. Excessive moisture may damage the electronic components. If your stimulator becomes wet, let it dry well before using it again.

SOME ADVICE ABOUT TRAVELING

Transport and store the stimulator in the travel case provided. This reduces the risk of damage. Airport x-ray machines will not harm the unit. You should register the unit at a customs office if you plan to take the unit out of the country. You will be provided with a letter of medical necessity to explain the purpose and use of the stimulator and your need to carry it with you.

APPENDIX IX

Patients Stimulation Log Sheet

Patients Daily Stimulation Log   (Weeks 1-5)
For questions, please call 343-6111 ext. 2606, ask for Carrie.

Name: _____   Doctor: _____

Date of Surgery: _____   Date of First Stimulation: _____

Week	Day	Hours of Stimulation	Intensity	Equipment Problems	Skin Problems	Comments
1						
1						
1						
1						
1						
1						
1						
2						
2						
2						
2						
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Describe equipment problems and date of occurences:

_____

_____

Describe skin problems and date of occurences:

_____

_____

Date(s) of battery change:

_____

_____

_____

APPENDIX X

Statistical Analysis of Force, EMG and MRI Data

Force Variables
Average values of the standardized measurements at pre-operation, 6 and 12 weeks
for the control and treatment groups separately and for two speeds separately.

--------------------------------- SPEED=60 VARIABLE=PT_ROM ---------------------------------

VARIABLE	GROUP	PRE-OPERATIVE MEASUREMENT	6 WEEK MEASUREMENT	12 WEEK MEASUREMENT
PT_ROM	CONTROL	0.86928	0.37908	0.53255
PT_ROM	TES	0.87185	0.46957	0.59338

--------------------------------- SPEED=60 VARIABLE=ttp_ms ---------------------------------

VARIABLE	GROUP	PRE-OPERATIVE MEASUREMENT	6 WEEK MEASUREMENT	12 WEEK MEASUREMENT
ttp_ms	CONTROL	1.24075	2.05913	1.93852
ttp_ms	TES	1.05575	0.94755	1.04321

--------------------------------- SPEED=60 VARIABLE=work_rom ---------------------------------

VARIABLE	GROUP	PRE-OPERATIVE MEASUREMENT	6 WEEK MEASUREMENT	12 WEEK MEASUREMENT
work_rom	CONTROL	0.81856	0.47202	0.63022
work_rom	TES	0.87514	0.45901	0.80572

--------------------------------- SPEED=60 VARIABLE=mpow_rom ---------------------------------

VARIABLE	GROUP	PRE-OPERATIVE MEASUREMENT	6 WEEK MEASUREMENT	12 WEEK MEASUREMENT
mpow_rom	CONTROL	0.87256	0.51495	0.66997
mpow_rom	TES	0.87534	0.47707	0.79999

--------------------------------- SPEED=180 VARIABLE=PT_ROM ---------------------------------

VARIABLE	GROUP	PRE-OPERATIVE MEASUREMENT	6 WEEK MEASUREMENT	12 WEEK MEASUREMENT
PT_ROM	CONTROL	0.83988	0.36603	0.54243
PT_ROM	TES	0.92325	0.50247	0.66994

--------------------------------- SPEED=180 VARIABLE=ttp_ms ---------------------------------

VARIABLE	GROUP	PRE-OPERATIVE MEASUREMENT	6 WEEK MEASUREMENT	12 WEEK MEASUREMENT
ttp_ms	CONTROL	1.29521	36.7154	6.12024
ttp_ms	TES	3.43301	3.8111	3.91140

------------------------------ SPEED=180 VARIABLE=work_rom ------------------------------

VARIABLE	GROUP	PRE-OPERATIVE MEASUREMENT	6 WEEK MEASUREMENT	12 WEEK MEASUREMENT
work_rom	CONTROL	0.73593	0.30130	0.48133
work_rom	TES	0.87187	0.41126	0.57736

------------------------------ SPEED=180 VARIABLE=mpow_rom ------------------------------

VARIABLE	GROUP	PRE-OPERATIVE MEASUREMENT	6 WEEK MEASUREMENT	12 WEEK MEASUREMENT
mpow_rom	CONTROL	0.74391	0.30293	0.45502
mpow_rom	TES	0.88049	0.41830	0.59487

SURGERY EFFECT ON THE FORCE VARIABLES

------------------------------ SPEED=60 MEASUREMENT=PT_ROM ------------------------------

MEASUREMENT	GROUP	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
PT_ROM	CONTROL	TIME Week6 - Week0	-0.490	0.040	-12.137	0.000
PT_ROM	CONTROL	TIME Week12 - Week6	0.153	0.040	3.800	0.001
PT_ROM	CONTROL	TIME Week12 - Week0	-0.337	0.040	-8.337	0.000

------------------------------ SPEED=60 MEASUREMENT=TTP_MS ------------------------------

MEASUREMENT	GROUP	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
TTP_MS	CONTROL	TIME Week6 - Week0	0.818	0.418	1.960	0.063
TTP_MS	CONTROL	TIME Week12 - Week6	-0.121	0.418	-0.289	0.775
TTP_MS	CONTROL	TIME Week12 - Week0	0.698	0.418	1.671	0.109

------------------------------ SPEED=60 MEASUREMENT=WORK_ROM ------------------------------

MEASUREMENT	GROUP	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
WORK_ROM	CONTROL	TIME Week6 - Week0	-0.347	0.063	-5.521	0.000
WORK_ROM	CONTROL	TIME Week12 - Week6	0.158	0.063	2.520	0.019
WORK_ROM	CONTROL	TIME Week12 - Week0	-0.188	0.063	-3.001	0.007

------------------------------ SPEED=60 MEASUREMENT=MPOW_ROM ------------------------------

MEASUREMENT	GROUP	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
MPOW_ROM	CONTROL	TIME Week6 - Week0	-0.358	0.064	-5.554	0.000
POW_ROM	CONTROL	TIME Week12 - Week6	0.155	0.064	2.408	0.025
POW_ROM	CONTROL	TIME Week12 - Week0	-0.203	0.064	-3.147	0.005

```
--------------------------- SPEED=180 MEASUREMENT=PT_ROM ---------------------------
```

MEASUREMENT	GROUP	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
PT_ROM	CONTROL	TIME Week6 - Week0	-0.474	0.061	-7.715	0.000
PT_ROM	CONTROL	TIME Week12 - Week6	0.176	0.061	2.872	0.009
PT_ROM	CONTROL	TIME Week12 - Week0	-0.297	0.061	-4.843	0.000

```
--------------------------- SPEED=180 MEASUREMENT=TTP_MS ---------------------------
```

MEASUREMENT	GROUP	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
TTP_MS	CONTROL	TIME Week6 - Week0	35.420	26.447	1.339	0.194
TTP_MS	CONTROL	TIME Week12 - Week6	-30.595	26.447	-1.157	0.260
TTP_MS	CONTROL	TIME Week12 - Week0	4.825	26.447	0.182	0.857

```
--------------------------- SPEED=180 MEASUREMENT=WORK_ROM ---------------------------
```

MEASUREMENT	GROUP	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
WORK_ROM	CONTROL	TIME Week6 - Week0	-0.435	0.083	-5.218	0.000
WORK_ROM	CONTROL	TIME Week12 - Week6	0.180	0.083	2.162	0.042
WORK_ROM	CONTROL	TIME Week12 - Week0	-0.255	0.083	-3.057	0.006

```
--------------------------- SPEED=180 MEASUREMENT=MPOW_ROM ---------------------------
```

MEASUREMENT	GROUP	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
MPOW_ROM	CONTROL	TIME Week6 - Week0	-0.441	0.084	-5.260	0.000
MPOW_ROM	CONTROL	TIME Week12 - Week6	0.152	0.084	1.814	0.083
MPOW_ROM	CONTROL	TIME Week12 - Week0	-0.289	0.084	-3.446	0.002

ANALYSIS FOR THE FORCE VARIABLES

```
--------------------------- SPEED=60 MEASUREMENT=PT_ROM ---------------------------
```

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
PT_ROM	PREOP TO 6 WEEKS	0.088	0.060	1.474	0.148
PT_ROM	QUADRATIC(See Text)	0.059	0.052	1.138	0.261

```
--------------------------- SPEED=60 MEASUREMENT=TTP_MS ---------------------------
```

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
TTP_MS	PREOP TO 6 WEEKS	-0.927	0.464	-1.997	0.052
TTP_MS	QUADRATIC(See Text)	-0.571	0.402	-1.422	0.162

```
--------------------------- SPEED=60 MEASUREMENT=MPOW_ROM ---------------------------
```

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
MPOW_ROM	PREOP TO 6 WEEKS	-0.041	0.139	-0.292	0.772
MPOW_ROM	QUADRATIC(See Text)	-0.104	0.121	-0.864	0.392

--------------------------- SPEED=60 MEASUREMENT=WORK_ROM ----------------------------------

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
WORK_ROM	PREOP TO 6 WEEKS	-0.070	0.136	-0.511	0.612
WORK_ROM	QUADRATIC(See Text)	-0.129	0.118	-1.095	0.279

--------------------------- SPEED=180 MEASUREMENT=PT_ROM ----------------------------------

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
PT_ROM	PREOP TO 6 WEEKS	0.053	0.080	0.664	0.510
PT_ROM	QUADRATIC(See Text)	0.031	0.069	0.448	0.657

--------------------------- SPEED=180 MEASUREMENT=TTP_MS ----------------------------------

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
TTP_MS	PREOP TO 6 WEEKS	-35.042	26.452	-1.325	0.192
TTP_MS	QUADRATIC(See Text)	-32.869	22.908	-1.435	0.158

--------------------------- SPEED=180 MEASUREMENT=MPOW_ROM ----------------------------------

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
MPOW_ROM	PREOP TO 6 WEEKS	-0.021	0.106	-0.201	0.842
MPOW_ROM	QUADRATIC(See Text)	-0.023	0.092	-0.250	0.804

--------------------------- SPEED=180 MEASUREMENT=WORK_ROM ----------------------------------

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
WORK_ROM	PREOP TO 6 WEEKS	-0.026	0.105	-0.246	0.807
WORK_ROM	QUADRATIC(See Text)	-0.006	0.091	-0.066	0.948

EMG ACTIVITIES FROM THE VASTUS LATERALIS (VL)
Average values of the standardized measurements at pre-operation, 6 and 12 weeks
for the control and treatment groups separately and for two speeds separately.

--------------------------- SPEED=60 VARIABLE=MED ----------------------------------

	Pre-operative	6 week	12 week

VARIABLE	group	measurement	measurement	measurement
medp_102	CONTROL	0.93	0.78	0.90
medp_102	TES	0.93	0.82	0.81

------------------------- SPEED=60 VARIABLE=iemg_rom -------------------------

VARIABLE	group	Pre-operative measurement	6 week measurement	12 week measurement
iemg_rom	CONTROL	0.79	0.41	0.51
iemg_rom	TES	0.88	0.57	0.69

------------------------- SPEED=180 VARIABLE=MED -------------------------

VARIABLE	group	Pre-operative measurement	6 week measurement	12 week measurement
medp_102	CONTROL	0.92	0.73	0.87
medp_102	TES	0.97	0.75	0.77

------------------------- SPEED=180 VARIABLE=iemg_rom -------------------------

VARIABLE	group	Pre-operative measurement	6 week measurement	12 week measurement
iemg_rom	CONTROL	0.88	0.39	0.67
iemg_rom	TES	0.85	0.60	0.73

EMG ACTIVITIES FROM THE VASTUS MEDIALIS (VM)

Average values of the standardized measurements at pre-operation, 6 and 12 weeks for the control and treatment groups separately and for two speeds separately.

------------------------- SPEED=60 VARIABLE=MED -------------------------

VARIABLE	GROUP	Pre-operative measurement	6 week measurement	12 week measurement
medp_102	CONTROL	0.99	0.69	0.81
medp_102	TES	1.00	0.78	0.80

------------------------- SPEED=60 VARIABLE=iemg_rom -------------------------

VARIABLE	GROUP	Pre-operative measurement	6 week measurement	12 week measurement
iemg_rom	CONTROL	0.83	0.74	0.84
iemg_rom	TES	0.93	0.59	0.64

------------------------- SPEED=180 VARIABLE=MED -------------------------

VARIABLE	GROUP	Pre-operative measurement	6 week measurement	12 week measurement

medp_102	CONTROL	0.96	0.68	0.75
medp_102	TES	1.05	0.76	0.76

------------------------------ SPEED=180 VARIABLE=iemg_rom ------------------------------

VARIABLE	GROUP	Pre-operative measurement	6 week measurement	12 week measurement
iemg_rom	CONTROL	0.77	0.59	0.90
iemg_rom	TES	0.98	0.56	0.85

SURGERY EFFECT ON THE VL VARIABLES

------------------------------ SPEED=60 MEASUREMENT=MED ------------------------------

MEASUREMENT	group	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
MEDP_102	CONTROL	TIME Week6 - Week0	-0.151	0.043	-3.537	0.002
MEDP_102	CONTROL	TIME Week12 - Week6	0.115	0.043	2.687	0.013
MEDP_102	CONTROL	TIME Week12 - Week0	-0.036	0.043	-0.850	0.405

------------------------------ SPEED=60 MEASUREMENT=IEMG_ROM ------------------------------

MEASUREMENT	group	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
IEMG_ROM	CONTROL	TIME Week6 - Week0	-0.382	0.049	-7.811	0.000
IEMG_ROM	CONTROL	TIME Week12 - Week6	0.103	0.049	2.101	0.047
IEMG_ROM	CONTROL	TIME Week12 - Week0	-0.279	0.049	-5.710	0.000

------------------------------ SPEED=180 MEASUREMENT=MED ------------------------------

MEASUREMENT	group	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
MEDP_102	CONTROL	TIME Week6 - Week0	-0.190	0.061	-3.137	0.005
MEDP_102	CONTROL	TIME Week12 - Week6	0.140	0.061	2.306	0.031
MEDP_102	CONTROL	TIME Week12 - Week0	-0.050	0.061	-0.831	0.415

------------------------------ SPEED=180 MEASUREMENT=IEMG_ROM ------------------------------

MEASUREMENT	group	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
IEMG_ROM	CONTROL	TIME Week6 - Week0	-0.485	0.099	-4.918	0.000
IEMG_ROM	CONTROL	TIME Week12 - Week6	0.279	0.099	2.823	0.010
IEMG_ROM	CONTROL	TIME Week12 - Week0	-0.207	0.099	-2.095	0.048

SURGERY EFFECT ON THE VM VARIABLES

------------------------------ SPEED=60 MEASUREMENT=MED ------------------------------

MEASUREMENT	GROUP	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE

MEASUREMENT	GROUP	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
.EDP_102	CONTROL	TIME Week6 - Week0	-0.308	0.078	-3.967	0.001
MEDP_102	CONTROL	TIME Week12 - Week6	0.126	0.078	1.621	0.119
MEDP_102	CONTROL	TIME Week12 - Week0	-0.182	0.078	-2.347	0.028

---------------------------- SPEED=60 MEASUREMENT=IEMG_ROM ----------------------------------

MEASUREMENT	GROUP	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
IEMG_ROM	CONTROL	TIME Week6 - Week0	-0.093	0.103	-0.900	0.378
IEMG_ROM	CONTROL	TIME Week12 - Week6	0.101	0.103	0.981	0.337
IEMG_ROM	CONTROL	TIME Week12 - Week0	0.008	0.103	0.081	0.936

---------------------------- SPEED=180 MEASUREMENT=MED ----------------------------------

MEASUREMENT	GROUP	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
MEDP_102	CONTROL	TIME Week6 - Week0	-0.283	0.100	-2.820	0.010
MEDP_102	CONTROL	TIME Week12 - Week6	0.073	0.100	0.726	0.475
MEDP_102	CONTROL	TIME Week12 - Week0	-0.210	0.100	-2.093	0.048

---------------------------- SPEED=180 MEASUREMENT=IEMG_ROM ----------------------------------

MEASUREMENT	GROUP	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
IEMG_ROM	CONTROL	TIME Week6 - Week0	-0.177	0.149	-1.189	0.247
IEMG_ROM	CONTROL	TIME Week12 - Week6	0.313	0.149	2.101	0.047
IEMG_ROM	CONTROL	TIME Week12 - Week0	0.136	0.149	0.912	0.372

TEST OF SIGNIFICANCE FOR THE VL MEASUREMENTS

---------------------------- SPEED=60 MEASUREMENT=MED ----------------------------------

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
MEDP_102	PREOP TO 6 WEEKS	0.04	0.06	0.69	0.49
MEDP_102	QUADRATIC(See Text)	0.09	0.05	1.61	0.11

---------------------------- SPEED=60 MEASUREMENT=IEMG_ROM ----------------------------------

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
IEMG_ROM	PREOP TO 6 WEEKS	0.07	0.10	0.71	0.48
IEMG_ROM	QUADRATIC(See Text)	0.03	0.09	0.28	0.78

---------------------------- SPEED=180 MEASUREMENT=MED ----------------------------------

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
MEDP_102	PREOP TO 6 WEEKS	-0.02	0.08	-0.24	0.81
MEDP_102	QUADRATIC(See Text)	0.05	0.07	0.75	0.46

------------------------- SPEED=180 MEASUREMENT=IEMG_ROM -----------------------------

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
IEMG_ROM	PREOP TO 6 WEEKS	0.24	0.14	1.71	0.09
IEMG_ROM	QUADRATIC(See Text)	0.19	0.12	1.62	0.11

TEST OF SIGNIFICANCE OF THE VM MEASUREMENTS

------------------------- SPEED=60 MEASUREMENT=MED -----------------------------

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
MEDP_102	PREOP TO 6 WEEKS	0.09	0.09	1.02	0.31
MEDP_102	QUADRATIC(See Text)	0.10	0.08	1.29	0.20

------------------------- SPEED=60 MEASUREMENT=IEMG_ROM -----------------------------

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
IEMG_ROM	PREOP TO 6 WEEKS	-0.25	0.13	-1.96	0.06
IEMG_ROM	QUADRATIC(See Text)	-0.10	0.11	-0.88	0.38

------------------------- SPEED=180 MEASUREMENT=MEDP_102 -----------------------------

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
MEDP_102	PREOP TO 6 WEEKS	-0.01	0.11	-0.12	0.91
MEDP_102	QUADRATIC(See Text)	0.03	0.10	0.27	0.79

------------------------- SPEED=180 MEASUREMENT=MED -----------------------------

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
MEDP_NO	PREOP TO 6 WEEKS	-0.03	0.03	-1.21	0.23
MEDP_NO	QUADRATIC(See Text)	-0.01	0.02	-0.32	0.75

------------------------- SPEED=180 MEASUREMENT=IEMG_ROM -----------------------------

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
IEMG_ROM	PREOP TO 6 WEEKS	-0.24	0.19	-1.27	0.21
IEMG_ROM	QUADRATIC(See Text)	-0.11	0.16	-0.64	0.52

MEAN EMG

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------------------------ SPEED=60 VARIABLE=MEAN IEMG ROM -----------------------
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		PRE-OPERATIVE	6 WEEK	12 WEEK
VARIABLE	GROUP	MEASUREMENT	MEASUREMENT	MEASUREMENT
MEAN IEMG ROM	CONTROL	0.81	0.57	0.68
MEAN IEMG ROM	TES	0.91	0.58	0.67

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------------------------ SPEED=180 VARIABLE=MEAN IEMG ROM ----------------------
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		PRE-OPERATIVE	6 WEEK	12 WEEK
VARIABLE	GROUP	MEASUREMENT	MEASUREMENT	MEASUREMENT
MEAN IEMG ROM	CONTROL	0.82	0.49	0.79
MEAN IEMG ROM	TES	0.92	0.58	0.79

SURGERY EFFECT ON THE MEAN LEG EMG VARIABLES

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------------------------ SPEED=60 MEASUREMENT=MEAN IEMG ROM --------------------
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MEASUREMENT	group	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
MEAN IEMG ROM	CONTROL	TIME Week6 - Week0	-0.237	0.065	-3.668	0.001
MEAN IEMG ROM	CONTROL	TIME Week12 - Week6	0.102	0.065	1.578	0.129
MEAN IEMG ROM	CONTROL	TIME Week12 - Week0	-0.135	0.065	-2.091	0.048

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------------------------ SPEED=180 MEASUREMENT=MEAN IEMG ROM -------------------
```

MEASUREMENT	group	TIME COURSE	SURGERY EFFECT	STANDARD ERROR	T-VALUE	P_VALUE
MEAN IEMG ROM	CONTROL	TIME Week6 - Week0	-0.331	0.100	-3.312	0.003
MEAN IEMG ROM	CONTROL	TIME Week12 - Week6	0.296	0.100	2.957	0.007
MEAN IEMG ROM	CONTROL	TIME Week12 - Week0	-0.036	0.100	-0.355	0.726

Test of significance for MEAN EMG

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------------------------ SPEED=60 MEASUREMENT=MEAN IEMG ROM --------------------
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MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
MEAN IEMG ROM	PREOP TO 6 WEEKS	-0.088	0.086	-1.028	0.309
MEAN IEMG ROM	QUADRATIC(See Text)	-0.036	0.074	-0.486	0.629

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------------------------ SPEED=180 MEASUREMENT=MEAN IEMG ROM -------------------
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MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
MEAN IEMG ROM	PREOP TO 6 WEEKS	-0.002	0.129	-0.016	0.988
MEAN IEMG ROM	QUADRATIC(See Text)	0.044	0.112	0.396	0.694

SURGERY EFFECT ON MRI VARIABLES

GROUP	PARM	EST	SE	T-VALUE	P_VALUE	MEASURE
CONTROL	POST - PRE	0.058	0.195	0.299	0.777	SQF
CONTROL	POST - PRE	-0.231	0.108	-2.135	0.070	SVM

Analysis for the MRI variables

MEASUREMENT	GROUP	PRE OPERATION MEAN	POST OPERATION MEAN
SQF	CONTROL	1.04	1.10
	TES	1.21	1.01
SVM	CONTROL	1.42	1.19
	TES	1.42	1.06

TEST OF SIGNIFICANCE OF MRI MEASUREMENTS

MEASUREMENT	TIME COURSE	TREATMENT MINUS CONTROL	STANDARD ERROR	T-VALUE	P_VALUE
SQF	PRE - POST	-0.26	0.20	1.26	0.24
SVM	PRE - POST	-0.14	0.16	0.83	0.43

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