COUNTERMEASURES AGAINST SHORT-TERM DISUSE ATROPHY

LOW VOLUME RESISTANCE EXERCISE PREVENTS LOSS OF MUSCLE MASS AND FUNCTION DURING 14 DAYS OF KNEE IMMOBILIZATION

By

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ABSTRACT

We aimed to determine the effectiveness of a low volume of high-intensity resistance exercise, alone (EX) or in combination with a whey protein supplement (WHY+EX), on prevention of muscle mass and strength loss following 14 days of knee immobilization in humans. Seventeen recreationally active (i.e., exercise $\leq$ 2-3 $d \cdot wk^{-1}$) participants (23.9±5.0 yr; BMI = 25.4±3.6 $kg \cdot m^{-2}$) were divided into three groups: exercise (EX; n=6), whey protein supplementation with exercise (WHY+EX; n=6), and control (CON; n=5). All subjects wore a knee-immobilization brace such that one leg was completely non-weight bearing for 14d. The resistance exercise (RE) were performed unilaterally and consisted of one set of ten repetitions of leg press (with plantar flexion at full extension), knee extension, and seated calf raises every other day during the 14d immobilization period, at 80% of one repetition maximum. Subjects in the WHY+EX group consumed two 30g boluses of whey protein daily while EX and CON consumed isocaloric carbohydrate beverages. Immobilization induced a significant reduction ($p<0.05$) in thigh cross-sectional area (CSA), isometric knee extensor strength, and isometric plantar flexion strength in CON but not in EX and WHY+EX. There were significant losses in lower leg CSA for all three groups, with a tendency for losses to be mitigated in both the EX and WHY+EX groups versus CON ($p=0.065$). The two constituent muscles of the triceps surae muscle group showed a differential response to the RE training with the gastrocnemius showing reductions in CSA almost uniformly across the three groups whereas soleus CSA was significantly reduced in the CON ($p<0.05$) but not in the EX and WHY+EX groups. We conclude that a relatively low volume of high-intensity resistance exercise is an effective countermeasure against atrophy of the thigh and the soleus muscle, as well as against knee extension and plantar flexion strength loss during 14d of leg immobilization. As a countermeasure to immobilization, there is no additional benefit of consumption of a daily whey protein supplement in combination with resistance exercise in maintaining muscle mass or strength.

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GLOSSARY OF TERMS

BMI – body mass index
CSA – cross-sectional area
CON – control group
DEXA – dual energy X-ray absorptiometry
DR – dietary record
EX – resistance exercise group
Gast – gastrocnemius muscle
HRt – half relaxation time
iEMG – integrated electromyography (surface)
ITT – interpolated twitch torque
KE – knee extension
EAA – essential amino acids
MRI – magnetic resonance imaging
MUA – motor unit activation
MVC – maximal voluntary contraction
NEAA – non-essential amino acids
PF – plantar flexion
PTT – peak twitch torque
QF – quadriceps femoris muscle
RE – resistance exercise
Rt – rise time
Sol – soleus muscle
TS – triceps surae muscle group
VL – vastus lateralis muscle
VM – vastus medialis muscle
WHY+EX – whey protein plus resistance exercise group

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CHAPTER I

1. INTRODUCTION

Skeletal muscle atrophy refers to the loss of muscle protein that occurs as a result of chronic unloading, chronic inactivity (disuse is considered here as synonymous with inactivity), or catabolic disease. There are several models of non-disease atrophy that have been well characterized, including unilateral lower limb immobilization (IM) and its close cousin unilateral lower limb suspension (ULLS), bed rest (BR), and space flight (SF). The effects of these different models of inactivity on muscle cross-sectional area (CSA) and isometric maximal voluntary contraction (MVC) strength have been well described in the literature and will be discussed in this review.

Resistance exercise and amino acids are independent stimulators of muscle protein synthesis (MPS) (Rennie *et al.*, 2004;Phillips *et al.*, 2005). In healthy humans, resistance exercise training in combination with sufficient provision of dietary amino acids results in a synergistic effect on MPS and optimizes the capacity to accrue new muscle tissue. It is not surprising then that both resistance exercise and nutritional supplementation have been employed as countermeasures during periods of disuse in an effort to mitigate the lean mass and strength loss that result. To date, however, most efforts to ameliorate atrophy through resistive exercise have used classic resistance training-type paradigms and no effort has been made to establish a minimally effective dose of resistive exercise that could offset atrophy and functional deconditioning of the unloaded muscle. Identifying countermeasures to atrophy that attenuate, if not prevent, muscle mass and function loss is important as even partial maintenance of muscle mass during periods of disuse-induced muscle loss may have a profound positive influence on rehabilitation time (Hortobagyi *et al.*, 2000).

2. REGULATION OF PROTEIN TURNOVER

The regulation of skeletal muscle mass is a dynamic process that can be affected by such things as exercise, nutrition, and disease. Muscle protein mass gain or loss is defined by the difference between the processes of muscle protein synthesis (MPS) and muscle protein breakdown (MPB). The algebraic difference, termed net protein balance (NPB), if chronically positive will ultimately translate into a net gain of muscle mass (hypertrophy) or, if negative, a net loss of muscle mass (atrophy). When skeletal muscle is chronically overloaded, as is the case with resistance exercise training, the result is a net positive balance or accretion of muscle mass. In situations of inactivity, for example spaceflight, bed-rest, and limb immobilization, a persistent net negative balance leads to a net loss of muscle mass (Adams *et al.*, 2003).

In healthy individuals between the ages of 18 and 40, the amount of muscle mass is generally unchanged over time, given that the individual is not resistance training and is consuming sufficient dietary protein since the positive NPB during the fed-state is equivalent to the fasted-state losses (Figure 1) (Phillips *et al.*, 2005).

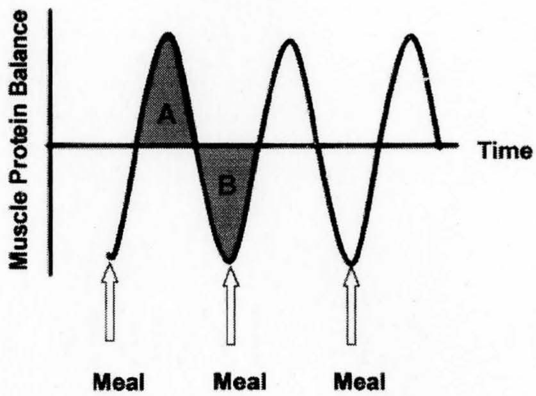

Figure 1: Normal fed-state gains and fasted-state losses in skeletal muscle protein balance (synthesis minus breakdown) – adapted from (Phillips et al., 2005)

The stimulation of MPS by amino acid feeding is dependent on the type and amount of amino acids consumed. There is considerable evidence to suggest an exclusive role for essential amino acids (EAA) in stimulation of a positive net balance with little or no contribution from non-essential amino acids (NEAA) (Borsheim *et al.*, 2002;Miller *et al.*, 2003). One study compared post-exercise consumption of 40 grams of essential amino acids (EAA) or 40 grams of mixed amino acids (~20g of EAA and ~20g of NEAA) and found no difference in net balance following the consumption of these two drinks post-exercise (Tipton *et al.*, 1999). Moreover, Cuthbertson and colleagues found that myofibrillar protein fractional synthetic rate (FSR) was stimulated in healthy young men, with a dose of 10g of EAA with no further increase in FSR with a 20g dose of EAA (Cuthbertson *et al.*, 2005). Thus, skeletal muscle seems to be particularly sensitive to EAA in a dose-dependent manner up to a threshold, which, when exceeded does not result in an additional increase in MPS.

Resistance exercise increases MPS and MPB such that muscle net balance is less negative than at rest; however, balance only becomes positive with the provision of amino acids (Phillips *et al.*, 2005). It has been shown that amino acid infusion post-exercise effectively increases FSR (>200%) and attenuates the exercise-induced increase in FBR (Biolo *et al.*, 1997); a similar result is seen when amino acids are consumed (Tipton *et al.*, 1999). Finally, when resistance exercise is accompanied with adequate amino acid provision, there is a synergistic effect on NPB such that it becomes more positive than with amino acid provision in the rested state or exercise alone (Figure 2) (Phillips *et al.*, 1997).

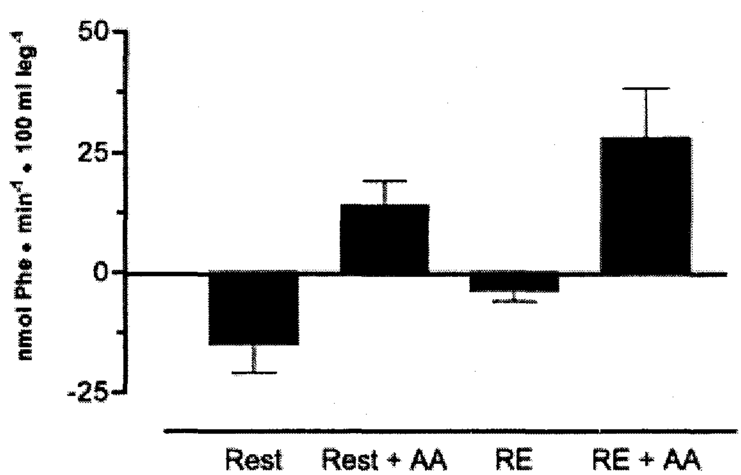

Figure 2: Influences of amino acids at rest, performance of resistance exercise, and post-exercise consumption of amino acids on net muscle protein balance – adapted from (Phillips *et al.*, 1997).

The importance of timing of the protein provision with resistance exercise has yielded conflicting results. When young males were given 6 g of EAA with 35 g of CHO (AA/CHO) at either one hour or three hours post-exercise, there were no differences in synthesis or net balance between the two time points (Rasmussen *et al.*, 2000). This suggests that there is approximately a three hour time frame following exercise in which a protein and carbohydrate beverage can be consumed to elicit the maximal positive muscle protein balance. In the elderly, it has been proposed that a potential limitation in the absorption of protein in the gut may delay the subsequent hyperaminoacidemia and result in lower net protein balance (Rasmussen *et al.*, 2002). Therefore, there appears to be a shorter window for post-exercise consumption of protein for optimal net balance in the elderly (Esmarck *et al.*, 2001). An evaluation of long-term (10-14 wks) resistance training studies reveals that the provision of amino acids or protein during the post-exercise period is important for maximally supporting muscle hypertrophy in healthy individuals (Esmarck *et al.*, 2001;Cribb & Hayes, 2006;Andersen *et al.*, 2005); Hartman *et al.*, In Press; Tang *et al.*, In Press).

3. NON-DISEASE MODELS OF DISUSE ATROPHY IN HUMANS

Muscle atrophy is characterized by a decrease in muscle CSA, which can be measured either at the muscle fibre or the whole muscle level. Skeletal muscle is essential to locomotion and a reduction in muscle mass can have profound effects on rehabilitation following a period of inactivity (Hortobagyi *et al.*, 2000). Moreover, since muscle CSA is directly proportional to strength, muscle wasting reduces strength which consequently increases frailty and may hinder quality of life in the aged.

Atrophy associated with microgravity (i.e., spaceflight) is the benchmark that many non-disease models of atrophy attempt to emulate. The data available from spaceflight remains sparse and lack of controls for activity level during spaceflight have resulted in high inter-subject variability (Fitts *et al.*, 2000). Many groups have used data from ground-based models of atrophy to make inferences regarding spaceflight due to the assumed homology in the mechanisms responsible for the alterations in skeletal muscle. However, it should be noted that spaceflight-induced changes in muscle mass and function may be unique to microgravity environments and it remains somewhat controversial whether ground-based models accurately describe the physiological adaptations seen with spaceflight (Widrick *et al.*, 2002;Adams, 2002).

Several ground based non-disease models been developed to study disuse atrophy in humans. Unilateral lower limb suspension (ULLS), limb immobilization (IM), and bed rest (BR) are all ground-based models of atrophy effective at inducing changes in muscle morphology and function (Adams *et al.*, 2003). These models aim to simulate inactivity/disuse/unloading and can be used to investigate non-humoral (i.e., hormone- or inflammatory-mediated) or non-disease models of atrophy. A similar degree of atrophy results from each of the three ground-based models of disuse (ULLS, IM and BR); moreover, the extent of spaceflight induced atrophy is comparable to that seen in ground-based models. Thus, it has been suggested that each of these models is appropriate for studying the effects of unloading on muscle morphology (Adams *et al.*, 2003).

3.1. Limb Immobilization

Limb immobilization usually refers to brace-mediated (Deschenes *et al.*, 2002;Yasuda *et al.*, 2005) or limb casting (Thom *et al.*, 2001;Jones *et al.*, 2004;Greenhaff, 2006). Each of these models effectively renders a joint (commonly the knee joint) immobile at a flexed angle, and eliminates the shortening or lengthening of the muscle. In the case of lower leg immobilization, crutches are used to allow for ambulation while remaining non-weight bearing on the immobilized leg.

3.2. Unilateral Lower Limb Suspension

Unilateral lower limb suspension (ULLS) is a similar model of unloading to limb immobilization. The most notable difference is the mobility of the knee joint (Adams *et al.*, 2003). Previously, two distinct models of ULLS have been employed. The first model of ULLS utilizes a support strap to suspend the limb, while the second model utilizes a high platform shoe on the contra-lateral limb (Figure 5). Both models effectively render one leg non-weight bearing (Adams *et al.*, 2003).

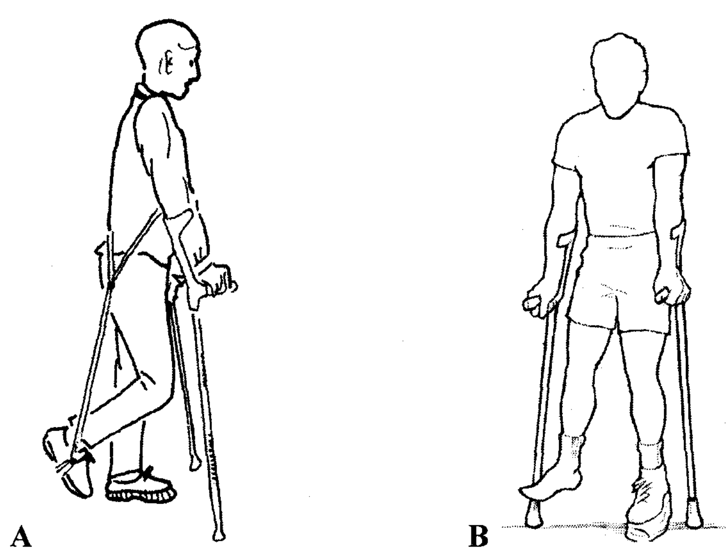

Figure 3: Schematic of unilateral lower limb suspension utilizing the strap (A) (Berg *et al.*, 1991) and the high platform shoe (B) (Tesch *et al.*, 2004)

The platform shoe model allows the leg to remain in the anatomical position at all times whereas the support strap method holds the knee joint in a slightly flexed position. As with limb immobilization, ambulatory activity is performed with the assistance of crutches.

3.3. Bed Rest

Bed rest is commonly used as the most applicable ground-based model to make inferences regarding microgravity-induced changes in muscle. It has been suggested that head-down tilt bed rest most accurately simulates the cardiovascular and musculoskeletal system adaptations to spaceflight (Adams *et al.*, 2003). Furthermore, the advantage to bed rest is the complete unweighting of antigravity postural muscles and a significant reduction in energy expenditure; both of these conditions are analogous to those experienced in the spaceflight environment (Adams *et al.*, 2003). Additionally, bed rest studies are conducted under strict supervision which ensures compliance and generally allows for control over dietary intakes; these two aspects are more difficult to control in other models of disuse.

3.4. Spaceflight

The physiological adaptations to a microgravity environment have become an area of increasing interest in recent years. One of the goals of research for life on the International Space Station (ISS) is to study the effects of spaceflight on muscle mass and function. Ultimately, the successful implementation of effective countermeasures, both resistance exercise and nutritional, will aim to ameliorate the health detriments consistent with space travel (Fitts *et al.*, 2000). Important considerations when evaluating the practicality of countermeasures are that muscles with antigravity functions experience chronic unloading and therefore may undergo more extensive atrophy (Adams *et al.*, 2003). Astronauts are also often in negative energy balance due to spaceflight-induced loss of appetite and there is typically limited time for performance of resistance exercise activities; these two conditions contribute to reductions in muscle mass (Adams *et al.*, 2003). Due to the limited size of spaceflight crews and their demanding schedules, very few spaceflight missions have allocated resources to identify the physiological adaptations to spaceflight (Adams *et al.*, 2003).

While ground-based models of atrophy remain practical alternatives to true microgravity, it is important to recognize that the effect of spaceflight on fibre diameter and strength are thought to be greater than in bed rest, so one must be cautious when using ground-based models to make inferences regarding spaceflight-induced skeletal muscle changes (Widrick *et al.*, 1999;Widrick *et al.*, 1997) .

4. EFFECTS OF MUSCLE DISUSE ON WHOLE MUSCLE MORPHOLOGY

4.1. Cross-sectional Area of Quadriceps Femoris

Lower limb unloading causes a reduction in the CSA of the quadriceps femoris muscle group, as shown in bed rest (Akima *et al.*, 2000;Kawakami *et al.*, 2001), unilateral lower limb suspension (ULLS) (Adams *et al.*, 1994;Ploutz-Snyder *et al.*, 1995;Schulze *et al.*, 2002), and knee immobilization/casting (IM) (Thom *et al.*, 2001;Yasuda *et al.*, 2005). The highest rate of muscle mass loss is thought to occur during the initial 1-3 weeks of disuse. It has been postulated that there is a rapid onset of atrophy, but with persistent unloading the process reaches a nadir (Bamman *et al.*, 1998;Mulder *et al.*, 2006). The loss of muscle volume during bed rest has been shown to be greatest in the first four weeks (Fitts *et al.*, 2007). Moreover, Alkner and Tesch (2004) showed that the rate of muscle loss in the first 29 days of 90 days of bed rest was approximately twice that of the final 60 days (Alkner & Tesch, 2004a). These data suggest that regardless of cellular processes that are mediating the disuse-induced atrophy, they are acting rapidly and early and then are adaptively downregulated with time. The slowing of atrophy is expected when you consider that no limb muscle mass would remain after 10-12 weeks if the rate of muscle protein loss seen early on continued during the entire disuse period. Even in models of human skeletal muscle 'paralysis' some degree of muscle remains.

Table I (A, B, and C) summarizes previous studies that have investigated changes in muscle CSA and strength following unilateral lower limb suspension (ULLS), knee immobilization (IM) or bed rest (BR) for the quadriceps femoris and triceps surae muscle groups.

4.2. Cross-sectional Area of Triceps Surae

Unloading-induced muscle mass losses in the triceps surae muscle group have been shown to exceed that of the quadriceps femoris muscle group during lower leg unloading or bed rest (Akima *et al.*, 2000;Alkner & Tesch, 2004a;Alkner & Tesch, 2004b). Intuitively, this makes sense due to the postural, anti-gravity function of the muscles in the lower leg, specifically the soleus muscle. The removal of the tonic stimulus of gravity by unweighting may result in a greater degree of atrophy compared to the quadriceps femoris because triceps surae muscles are more involved in day-to-day life and locomotion activity (Ericson *et al.*, 1986). However, evidence to support greater loss of muscle CSA in the calf versus the thigh remains inconclusive due to a limited number of studies designed to evaluate both muscle groups. Comparisons across studies are difficult to reconcile due to differences in participant activity level, duration of disuse period, and the model of unweighting. Muscle group-dependent responses to resistance exercise have been documented and are presented in Section 6.1 of this review.

Table I A. Summary of cross-sectional area and strength changes in the lower limb following a period of disuse (1991-2000)

Reference	Unweighting Model	Participants	Duration (days)	Findings (% decrease in CSA†)	Findings (% decrease in MVC†)
Berg et al. 1991	ULLS	6 males	28	7% (Q.F.)	22% (K.E.)
Dudley et al. 1992	ULLS	5 males, 3 females	30	NR	21% (K.E.)
Hather et al. 1992	ULLS	5 males, 3 females	42	16% (Q.F.)	NR
Adams et al. 1994	ULLS	10 males	16	8% (Q.F.)	12% (K.E.)
Tesch et al. 1994	ULLS	4 males, 3 females	35	14% (Q.F.)	20% (K.E.)
Ploutz-Snyder et al. 1995	ULLS	4 males, 3 females	35	14% (Q.F.)	20% (K.E.)
Berg et al. 1996	ULLS	10 males	10	NR	13% (K.E.)
Bamman et al. 1997	BR	8 males	14	NR	13% (P.F.)
Berg et al. 1997	BR	7 males	30	14% (Q.F.)	25-30% (K.E.)
Bamman et al. 1998	BR	8 males	14	16% (Q.F. Myofibre CSA)	15% (K.E.)
Akima et al. 2000	BR	4 males	20	7.8% (Q.F. PCSA) 12.8% (T.S. PCSA)	16% (K.E.)¥

ULLS: unilateral lower-limb suspension, IM: knee immobilization, BR: 6° head-down-tilt bed rest; CSA: cross-sectional area, PCSA: physiological CSA; MVC: maximal voluntary contraction; Q.F.: quadriceps femoris muscle group, T.S.: triceps surae muscle group; K.E.: knee extension strength, P.F.: plantar flexor strength; NR: not reported; ¥ non-significant result; † unless otherwise specified

Table I B. Summary of cross-sectional area and strength changes in the lower limb following a period of disuse (2000-2004)

Reference	Unweighting Model	Participants	Duration (days)	Findings (% decrease in CSA†)	Findings (% decrease in MVC†)
Hortobagyi et al. 2000	IM	36 males and females	21	~ 11% (myofibre)	48% (K.E.)
Akima et al. 2001	BR	10	20	7% (Q.F. PCSA); 11.9% (T.S. PCSA)	NR
Hespel et al. 2001	IM	13 males, 9 females	14	10% (Q.F.)	23% (K.E.)
Kawakami et al. 2001	BR	4 males	20	8% (Q.F. PCSA)	11% (K.E.)
Thom et al. 2001	ULLS	8 females	10	11.8% (Q.F.)	42% (K.E. 1RM)
Deschenes et al. 2002	IM	6 males, 4 females	14	0% (Q.F.)	22% (K.E.)
Shulze et al. 2002	ULLS	8 males	21	7% (Q.F.); 7% (T.S.)	17% (K.E.); 17% (P.F.)
Akima et al. 2003	BR	6 males	20	13% (T.S. PCSA)	9.4% (P.F.)¥
Alkner et al. 2004	BR	9 males	29	10% (Q.F. Volume); 16% (T.S. Volume)	NR
Alkner et al. 2004	BR	9 males	90	18% (Q.F. Volume); 29% (T.S. Volume)	31-60% (K.E.); 37-56% (P.F.)

ULLS: unilateral lower-limb suspension, IM: knee immobilization, BR: 6° head-down-tilt bed rest; CSA: cross-sectional area, PCSA: physiological CSA; MVC: maximal voluntary contraction; Q.F.: quadriceps femoris muscle group, T.S.: triceps surae muscle group; K.E.: knee extension strength, P.F.: plantar flexor strength; NR: not reported; ¥ non-significant result; † unless otherwise specified

Table I C. Summary of cross-sectional area and strength changes in the lower limb following a period of disuse (2004-2007)

Reference	Unweighting Model	Participants	Duration (days)	Findings (% decrease in CSA†)	Findings (% decrease in MVC†)
Jones et al. 2004	IM	9 males	14	4.7% (Q.F.) (lean mass)	27% (K.E.)
Paddon-Jones et al. 2004	BR	6 males	28	~300g (leg lean mass) 1004 mm^2 (T.S.)	~17.5% (K.E. 1-RM)
Tesch et al. 2004	ULLS	11 males and females	35	8.8% (Q.F. Volume) 10.5% (T.S. Volume)	24% (K.E.)
Yasada et al. 2005	IM	13 males, 14 females	14	5.8% (Q.F.)	~ 15-20% (K.E.)
Mulder et al. 2006	BR	9 males	56	14.1% (Q.F.)	16.8% (K.E.)
Fitts et al. 2007	BR	5 males	28	0% (leg lean mass)	23% (K.E.)

ULLS: unilateral lower-limb suspension, IM: knee immobilization, BR: 6° head-down-tilt bed rest; CSA: cross-sectional area, PCSA: physiological CSA; MVC: maximal voluntary contraction; Q.F.: quadriceps femoris muscle group, T.S.: triceps surae muscle group; K.E.: knee extension strength, P.F.: plantar flexor strength; NR: not reported; ¥ non-significant result; † unless otherwise specified

4.3. Cross-sectional Area of Myofibres

There is evidence showing a reduction in myofibre CSA resulting from 6 weeks of ULLS (Hather *et al.*, 1992), and 2-3 weeks of IM (Veldhuizen *et al.*, 1993;Hortobagyi *et al.*, 2000;Yasuda *et al.*, 2005). Conversely, there are examples of non-significant fibre CSA reductions elicited by 2-3 weeks of ULLS (Adams *et al.*, 1994;Deschenes *et al.*, 2002) and 2 weeks of IM (Hespel *et al.*, 2001). These differences in the reported findings at the myofibre level may be attributed to the accuracy of the assessment of the fibre CSA and distribution. Specifically, the failure to see a reduction in fibre CSA could have been the result of inadequate subject sample size or a limited number of usable fibres in the histochemistry sample. In addition, in certain models, removal of the immobilization sling or brace was actually encouraged and subjects were instructed to stretch and move their immobilized limb (Adams *et al.*, 1994;Deschenes *et al.*, 2002). It is not clear what minimal amount of weight bearing/stretching needs to occur to offset atrophy, but it may be that in the previous studies that enough movement occurred to at least slow atrophy and prevent the full atrophic response.

The evidence regarding fibre-specific (i.e., type I versus type II) atrophy following disuse is inconclusive. Some groups suggest increased susceptibility to wasting in the lower leg (greater percentage of type I fibres) versus the thigh (higher percentage of type II fibres) (Akima *et al.*, 2000;Alkner & Tesch, 2004b). One study has shown that the bed rest-induced fibre atrophy was 2-3 fold higher in the soleus than in the vastus lateralis during high cortisol conditions meant to mimic spaceflight conditions (Fitts *et al.*, 2007). Moreover, the type II fibres of the soleus and vastus lateralis muscles atrophied 20 and 6%, respectively, while the type I fibres in the soleus and vastus lateralis muscles were reduced 9 and 3% respectively. These data suggest that the soleus is more susceptible to atrophy and that there is a more rapid onset of wasting in the soleus compared to the vastus lateralis muscle (Fitts *et al.*, 2007); however, this may be due less to the fibre content of the soleus and more to its role as a tonically-active postural muscle. Conversely, several studies support the contention that there is no fibre-specific atrophy from disuse (Hather *et al.*, 1992;Hortobagyi *et al.*, 2000;Yasuda *et al.*, 2005). It has been suggested, however, that fibre atrophy occurs independent of fibre type but depends on the pre-disuse area; that is the greater the fibre CSA, the more susceptible that fibre is to atrophy (Fitts *et al.*, 2000).

5. EFFECTS OF MUSCLE DISUSE ON MUSCLE FUNCTION

5.1. Muscle Strength

Strength decrements resulting from situations of disuse are commonly expressed as a percent decrease in maximal voluntary contraction (MVC). The reduction in strength depends on several factors, including: the muscle group tested, the duration of unweighting period, the mode of contraction used for strength testing (i.e., isometric or isokinetic), the joint angle, neuromuscular adaptations to the unloading, and the model of unweighting being utilized. Across the various unweighting models there are reductions of ~15% for knee extension strength following ten days of unloading (Berg & Tesch, 1996). When unweighting persists for 2-3 weeks, the strength on average continues to decrease to ~23% (Adams *et al.*, 1994;Hortobagyi *et al.*, 2000;Hespel *et al.*, 2001;Kawakami *et al.*, 2001;Deschenes *et al.*, 2002;Schulze *et al.*, 2002;Jones *et al.*, 2004). Finally, during long-term disuse (≥35 days), there is a strength impairment of, on average, 28% (Mulder *et al.*, 2006;Tesch *et al.*, 2004;Alkner & Tesch, 2004b;Berg *et al.*, 1997;Ploutz-Snyder *et al.*, 1995;Tesch *et al.*, 1994). One must be careful not to over-interpret these average strength losses at various durations of disuse as they incorporate data from a variety of unweighting models. However, these data do suggest that the rate of strength loss is largest in the initial 2-3 weeks of unloading.

Despite concerns that the anti-gravity, postural muscles (e.g., soleus) may be more susceptible to strength decrements during unloading than other muscle groups with a mixed fibre distribution (e.g., vastus muscles), data evaluating the functional deficit to the triceps surae muscle group are lacking. Few studies have evaluated the strength loss of the plantar flexor muscle group following a period of disuse. The average strength loss was 13% and 17% respectively for durations of 14 and 21 days of disuse (Bamman *et al.*, 1997;Schulze *et al.*, 2002). Following 90 days of bed rest, the plantar flexion strength impairment was comparable to that observed for knee extension, specifically, a decrease of approximately 45% (Alkner & Tesch, 2004b).

It has been postulated that since the strength improvements in the initial two weeks of resistance training occurs primarily as a result of improved neural recruitment that the strength decrements resulting from short-term unloading would result from impaired neural activation (Deschenes *et al.*, 2002). Although this rationale seems plausible, only Deschenes and colleagues (2002) reported no change in whole muscle CSA following short-term (14d) unloading. Other short-term unloading studies report reductions in quadriceps femoris CSA or lean mass on the order of 4.7% - 11.8% after 10-14 days of unilateral lower limb suspension or limb immobilization (Yasuda *et al.*, 2005;Jones *et al.*, 2004;Hespel *et al.*, 2001;Thom *et al.*, 2001). Muscle specific force (i.e., force/CSA) can be calculated to assess whether the strength decrement is proportional to the degree of atrophy. A reduction in muscle specific force would indicate that there is additional strength loss which may associated with a neuromuscular impairment and this result has been reported in short-term (14d) knee immobilization in females (Yasuda *et al.*, 2005).

5.2. Muscle Function

Electromyography (EMG) and percent motor-unit activation (% MUA) are commonly used to assess neuromuscular function. When muscle strength loss is not proportional to the muscle atrophy, one explanation is an alteration in one of these indices of muscle function. It has been suggested that during short-term disuse, strength loss is primarily due to muscle atrophy (Bamman *et al.*, 1997). Indeed, there was no change in maximal EMG following 10 days of ULLS (Berg & Tesch, 1996) or following 14 days of bed rest (Bamman *et al.*, 1998). Conversely, following 21 days of ULLS there was a significant reduction in maximal EMG for both vastus medialis (26%) and soleus (31%) (Schulze *et al.*, 2002). Interestingly, in this study (Schulze *et al.*, 2002) it was reported that a resistance exercise countermeasure was effective at mitigating the reduction in EMG. Alkner and colleagues (2004) found a reduction in maximal EMG following 90 days of bed rest, but also reported that the resistance exercise countermeasure group showed no changes following this bed rest period (Alkner & Tesch, 2004b). It has been suggested that a decrease in motor-unit recruitment following the disuse period was responsible for the decrease in maximal EMG, however, it was further speculated that impairment of neural activation can be easily prevented with brief muscle usage (Schulze *et al.*, 2002;Mulder *et al.*, 2006).

Furthermore, during 6 weeks of unloading, there are significant reductions in neural activation, as measured by maximal EMG, in the ULLS model (Dudley *et al.*, 1992) and the bed rest model (Berg *et al.*, 1997). Conversely, following 8 weeks of bed rest, there were no alterations in maximal voluntary activation level, as measured by percent motor unit activation (%MUA) (Mulder *et al.*, 2006). The %MUA is a technique that can be used to evaluate the central nervous systems ability to recruit all motor units for a given muscle group and fire them at a maximal frequency. Impairment in the %MUA would lead to compromised torque production during an MVC. When strength losses are disproportionate to the unloading-induced atrophy, this result would imply a neural-based strength loss, either central or via a mechanism intrinsic to the muscle. It is possible that the %MUA and EMG are unaltered following disuse, indicating maintenance of central neural drive. However, a reduction in muscle torque generating capacity in combination with unaltered muscle electrical activity implies an impairment in excitation-contraction (E-C) coupling (Keeton & Binder-Macleod, 2006).

There is evidence to support both no changes in neural activation following unloading (Berg & Tesch, 1996;Bamman *et al.*, 1997;Bamman *et al.*, 1998;Mulder *et al.*, 2006), as well as changes in neuromuscular function which result in loss of strength

(Kawakami *et al.*, 2001;Deschenes *et al.*, 2002;Schulze *et al.*, 2002;Alkner & Tesch, 2004b). Clearly, the influence of neuromuscular function on strength during unloading remains controversial and requires further study.

6. COUNTERMEASURES TO PREVENT ATROPHY AND STRENGH LOSS

6.1. Resistance Exercise Countermeasure

Lower limb unweighting and resistance exercise induce opposing skeletal muscle adaptations. Resistance training throughout an immobilization or disuse period has been utilized to ameliorate the losses of muscle mass and strength. Resistance exercise countermeasures are typically designed to provide maximal muscle loading in a minimal amount of time (Bamman *et al.*, 1997). Following an acute bout of resistance exercise, the muscle net balance (FSR minus FBR) increases and remains elevated at 48 hours post-exercise (Phillips *et al.*, 1997). Conversely, there was a reduction in resting muscle protein synthesis (MPS) following approximately 37 days of cast mediated lower leg immobilization. In agreement with this finding, a reduction in FSR was reported during 14 days (Ferrando *et al.*, 1997) and 28 days (Paddon-Jones *et al.*, 2006) of bed rest. Therefore, resistance exercise countermeasures are utilized to maintain the stimulation of MPS, which in turn attenuates the unloading-induced negative NPB.

An important consideration for implementing resistance exercise as a countermeasure of atrophy is the dose necessary to preserve muscle mass and function. To date, many studies have employed resistance training programs commonly utilized to promote hypertrophy in healthy individuals (Bamman *et al.*, 1997;Bamman *et al.*, 1998;Akima *et al.*, 2000;Akima *et al.*, 2001;Kawakami *et al.*, 2001;Akima *et al.*, 2003;Alkner & Tesch, 2004a), thus the minimal dose required to effectively protect muscle against wasting has not been established. Recently, Shulze and colleagues (Schulze *et al.*, 2002) were able to maintain quadriceps femoris and triceps surae muscle CSA with a relatively small dose of resistance exercise (2 x 5s MVC, 1 set at 40% 1-RM, and 2 sets at 80% 1-RM of knee extension and plantar flexion, every third day during 21 days of ULLS) which demonstrates the responsiveness of skeletal muscle to low volume resistance exercise during an unloading period. These data highlight the requirement for additional studies to investigate the minimal dose of exercise required to maintain lean mass, which will have implications for persons unable to perform large volumes of exercise during situations of muscle wasting.

There is a potential muscle group differential response to resistance exercise countermeasures. There is substantial evidence to support the effectiveness of resistance exercise training at maintaining the quadriceps femoris muscle group CSA and knee extensor MVC (Akima *et al.*, 2000;Kawakami *et al.*, 2001;Schulze *et al.*, 2002;Alkner & Tesch, 2004a;Mulder *et al.*, 2006) and even examples where the 'anti-atrophy' exercise protocol induced muscle hypertrophy (Akima *et al.*, 2001;Tesch *et al.*, 2004). Interestingly, the studies employing a resistance exercise protocol that was effective at full maintenance of quadriceps femoris muscle mass were ineffective at offsetting the atrophy of the triceps surae muscle group (Akima *et al.*, 2000;Akima *et al.*, 2001;Schulze *et al.*, 2002;Tesch *et al.*, 2004). Table II (A, B, and C) provides a summary of the literature to date that have employed resistance exercise or nutritional countermeasures during a period of disuse to ameliorate or prevent the losses in lean mass and strength.

Further questions regarding the effectiveness of a resistance exercise paradigm include whether strength is maintained in all modes of contraction (i.e., isometric and isokinetic) and across all joint angles. It has been speculated that the preservation of strength may only occur when the performance testing utilizes exercises analogous to those used during the training (Adams *et al.*, 2003). While this is potentially problematic when evaluating whether there was a true maintenance of strength, one study specifically evaluated plantar flexion deconditioning during 14 days of bed rest and found that the prevention of plantar flexor strength loss and the efficacy of training was seen in all contraction modes across all velocities (Bamman *et al.*, 1997).

The practical benefits of resistance training during unloading may lead to design of effective resistance exercise countermeasures for astronauts in microgravity, patients subject to bed rest, and more widely to anyone losing muscle mass due to inactivity. Admittedly, there is little relevance of resistance exercise countermeasures to those wearing a joint-immobilization cast, but the responsiveness of muscle to resistance exercise during chronic unloading highlights the importance of resistance exercise training during the rehabilitation period.

Table II A. Summary of cross-sectional area and strength changes in the lower limb following a period of disuse with a countermeasure

Reference	Unweighting Model	Participants	Duration (days)	Countermeasure (exercise/nutrition; frequency; amount)	Findings (% Change in CSA)	Findings (% Change in MVC)
Bamman et al. 1997	BR	8 males	14	Horizontal Dynamic Plantar Flexion; every second day; 5 sets x 6-10 repetitions (to failure)	NR	↔ * (P.F.)
Bamman et al. 1998	BR	8 males	14	Dynamic Leg Press; Every other day; 5 sets of 10 reps @ 80-85% 1-RM	↔ * (myofibre CSA)	↓ 13% (K.E.)
Akima et al. 2000	BR	5 males	20	Isometric Leg Press; Daily; 3sec x 30 repetitions	↔ * (Q.F.) ↓ 13% (T.S.)	↔ (K.E.) NR (P.F.)
Akima et al. 2001	BR	5 males	20	Dynamic Leg Press; 2 sessions daily; 3 sets of 10 reps @ 90% and 2 sets @40% to exhaustion	↑ 6.1%* (Q.F.) ↓ 11.6% (T.S.) (PCSA)	NR
Kawakami et al. 2001	BR	5 males	20	Isometric Leg Extension; Daily; 3s x 30reps with 3s rest between reps	↔ * (Q.F.) (PCSA)	↔ * (K.E.)

ULLS: unilateral lower-limb suspension, IM: knee immobilization, BR: 6° head-down-tilt bed rest; CSA: cross-sectional area, PCSA: physiological CSA, MVC: maximal voluntary contraction; Q.F.: quadriceps femoris; T.S.: triceps surae; K.E.: knee extension strength, P.F. plantar flexion strength; NR: not reported; * significant improvement vs. control group; ↔ no change, ↑ increase, ↓ decrease

Table II B. Summary of muscle size and strength changes in the lower limb following a period of disuse with a countermeasure

Reference	Unweighting Model	Participants	Duration (days)	Countermeasure (exercise/nutrition; frequency; amount)	Findings (% Change in CSA)	Findings (% Change in MVC)
Shulze et al. 2002	ULLS	8 males	21	Knee Extension and Plantar Flexion; Every third day; 10 isotonic @ 40%, 2 x 5-s MVC, 1 set of 10 isotonic @ 80%,1 set of 10 isotonic @ 80% to failure	↔ * (Q.F.) ↔ * (T.S.)	↔ * (K.E.) ↔ * (P.F.) (1-RM)
Akima et al. 2003	BR	6 males	20	Dynamic Leg Press and Plantar Flexion; 2 sessions daily for 16/20 days; 5 sets of 10 reps of leg press @ 70% (am) and 5 sets x 10 reps of plantar flexion @ 70% (pm)	↔ * (T.S.)	↔ (P.F.)
Alkner et al. 2004	BR	8 males	29	Supine Squats and Calf Press; every third day; squats - 4 sets x 7 reps and calf press (4 sets x 14 reps)	↔ * (Q.F.) ↓ 8% * (T.S.)	↔ (K.E.) ↔ (P.F.)
Alkner et al. 2004	BR	8 males	90	Supine Squats and Calf Press; every third day; squats - 4 sets x 7 reps and calf press (4 sets x 14 reps)	↔ * (Q.F.) ↓15% * (T.S.)	NR

ULLS: unilateral lower-limb suspension, IM: knee immobilization, BR: 6° head-down-tilt bed rest; CSA: cross-sectional area, PCSA: physiological CSA, MVC: maximal voluntary contraction; Q.F.: quadriceps femoris; T.S.: triceps surae; K.E.: knee extension strength, P.F. plantar flexion strength; NR: not reported; * significant improvement vs. control group; ↔ no change, ↑ increase, ↓ decrease

Table II C. Summary of cross-sectional area and strength changes in the lower limb following a period of disuse with a countermeasure

Reference	Unweighting Model	Participants	Duration (days)	Countermeasure (exercise/nutrition; frequency; amount)	Findings (% Change in CSA)	Findings (% Change in MVC)
Paddon-Jones et al. 2004	BR	7 males	28	Amino Acid (AA)/ Carbohydrate(CHO) Supplement; 3 times daily; 16.5g of essential AA/ 30g CHO	↔ * (leg lean mass) ↓ $491mm^2$(T.S.)	↓ * 8% (K.E.) (1-RM)
Tesch et al. 2004	ULLS	10 males & females	35	Flywheel Resistance Exercise; 2-3 times weekly; dynamic knee extension - 4 sets x 7 reps @ maximal plus sub-maximal warm-up	↑ 7.7% * (Q.F.) ↓ 11% (T.S.)	↔ * (K.E.)
Mulder et al. 2006	BR	9 males	56	Vibration System - supine squatting, heel & toe raises, explosive squatting; 2 times daily; 60-100s @ 2 times body weight	↔ * (Q.F.)	↔ * (K.E.)
Fitts et al. 2007	BR	5 males	28	Amino Acid (AA)/ Carbohydrate(CHO) Supplement; 3 times daily; 16.5g of essential AA/ 30g CHO	↔ * (leg lean mass)	↓ 11.1% * (K.E.)

ULLS: unilateral lower-limb suspension, IM: knee immobilization, BR: 6° head-down-tilt bed rest; CSA: cross-sectional area, PCSA: physiological CSA, MVC: maximal voluntary contraction; Q.F.: quadriceps femoris; T.S.: triceps surae; K.E.: knee extension strength, P.F. plantar flexion strength; NR: not reported; * significant improvement vs. control group; ↔ no change, ↑ increase, ↓ decrease

6.2. Nutritional Countermeasures

Recently, there has been considerable interest in evaluation of dietary manipulations and nutritional supplements as effective countermeasures of atrophy during periods of unloading. During situations in which resistance exercise cannot be performed, namely injury or disease-mediated bed rest, the prospect of partial maintenance of muscle mass by means of optimal nutrition could translate into shortened recovery times. In young, healthy individuals the net positive protein balance following a mixed meal is approximately equal to the net negative protein balance in the fasted state, such that the muscle mass is maintained over time (Phillips *et al.*, 2005). Moreover, it is only when resistance exercise is performed, in combination with amino acid provision, that net accretion of new proteins can be supported (Phillips *et al.*, 2005). However, the goal of a nutritional supplement during disuse atrophy is not to promote hypertrophy but rather to attenuate the loss of muscle.

In order to maximize the stimulus of nutrition on net protein balance, in the absence of resistance exercise, individuals undergoing muscle wasting should consider certain criteria for a supplement, which might include: an optimal dose (i.e., one that stimulated anabolism to its fullest extent and yet does not 'turn on' deamination and oxidative pathways for amino acid disposal) of high quality protein and it should be consumed at optimal times throughout the day. One such study has evaluated the effects of an amino acid (16.5g of EAAs) plus carbohydrate (30g of sucrose) (AA/CHO) supplement during prolonged bed rest. The AA/CHO supplement was given three times daily (762kJ, thrice daily), 1.5 hours following a mixed meal containing 14% protein.

The control group showed a significant loss in lower leg lean muscle mass (-346±130), as measured by dual energy X-ray absorptiometry (DEXA), while AA/CHO group showed no change (210±302) in lower limb lean mass after the bed rest (Paddon-Jones *et al.*, 2004). Interestingly, there were significant reductions in both the control and AA/CHO groups for CSA of the calf muscles as determined by magnetic resonance imaging (MRI). These data suggest that the maintenance of lean mass was exclusive to the thigh raising some interesting questions regarding whether muscle groups with different fibre type distributions have differing sensitivity to amino acids during a period of disuse.

In another study, with an identical duration of bed rest (28 days) and AA/CHO supplementation, there were no changes at the fibre level in either type I or type II fibre area (Fitts *et al.*, 2007). This indicates the necessity for a comprehensive evaluation of the use of DEXA, MRI and fibre histochemistry in accurately measuring skeletal muscle mass, area, and volume. Consequently, it is difficult to reconcile the differences in these findings when nutrition is used as a countermeasure of atrophy. If there is an effect of protein supplementation during disuse, the magnitude of the change would be subtle and likely difficult to detect.

Finally, the applicability of a nutritional supplement in maintenance of muscle mass during spaceflight poses a unique situation. Typically, crew members demonstrate elevated muscle protein breakdown due to inadequate energy intake and a negative caloric balance (Paddon-Jones *et al.*, 2005). The mechanism responsible for the net negative balance may be different than that proposed during bed rest, which is a depression of muscle protein synthesis (Fitts *et al.*, 2007;Ferrando *et al.*, 1996). This poses additional complications when using bed rest to make inferences regarding spaceflight. In addition, spaceflight-induced nausea and loss of appetite may contribute to a negative nitrogen balance which can affect muscle mass (Cena *et al.*, 2003).

7. SUMMARY

Several ground-based models of atrophy have been utilized to investigate changes in muscle morphology and function. Changes based on various models have often been compared to changes seen from spaceflight. The ground-based models yield similar degrees of atrophy to that seen with spaceflight; however, regardless of the type of unloading, there is considerable variability between subjects with some demonstrating more atrophy and functional loss than others (Fitts *et al.*, 2007). This may not be surprising given that there is also considerable variability in the degree of hypertrophy seen with resistance exercise programs (Hubal *et al.*, 2005).

Both short term and long duration disuse yield similar atrophy and strength loss for the quadriceps femoris and triceps surae muscle groups. However one notable difference appears to be the responsiveness of the two muscle groups to resistance exercise and nutritional countermeasures. Studies have provided evidence to support full maintenance of quadriceps femoris muscle mass with various doses of resistance exercise but the same is not true for the triceps surae muscle group. Some studies have successfully ameliorated the losses in calf muscle mass while others have shown no effect of a resistance exercise countermeasure on calf muscle mass or strength. These discrepancies demonstrate the need for a study designed to directly compare the differences between the thigh and calf, and the responsiveness to a resistance exercise countermeasure. A comprehensive study utilizing DEXA, MRI and fibre histochemistry to evaluate thigh and calf morphological changes in combination with various indices of knee extensor and plantar flexion muscle function has not been conducted. Furthermore, it is difficult to reconcile differences in the literature due to various durations of unloading, differing measures of muscle performance, and various models of unweighting.

8. RATIONALE FOR RESEARCH

The objective of most non-disease atrophy research has focused on describing the morphological and functional changes to skeletal muscle with unloading. More recently, the objectives have shifted and the aim to develop effective countermeasures has become a priority. Resistance exercise and amino acid feeding are potent and apparently independent stimulators of muscle protein synthesis. It is not known, however, to what extent a minimal dose of exercise, or exercise and nutritional supplementation in combination may be used as countermeasures against losses of muscle mass and strength during disuse atrophy. The successful development of such resistance exercise and nutritional countermeasures will have implications for patients exposed to bed rest, long duration spaceflight missions, and more widely, to any persons experiencing loss of muscle mass due to inactivity.

9. STATEMENT OF RESEARCH QUESTION AND HYPOTHESIS

The aim of this study was to determine the effectiveness of low volume, high-intensity resistance exercise, alone or in combination with a whey protein supplement, on muscle mass and strength following 14 days of unilateral knee immobilization in humans. Despite the fact that published studies to date (Bamman *et al.*, 1997;Bamman *et al.*, 1998;Akima *et al.*, 2000;Akima *et al.*, 2001;Kawakami *et al.*, 2001;Akima *et al.*, 2003;Alkner & Tesch, 2004a) have relatively large volumes of resistance exercise, many modeled on sets and repetitions used to promote hypertrophy, we chose a smaller volume of exercise. The rationale for this was to see if a relatively minimal dose of intense resistive contractions could offset atrophy with the recognition that, for example, anti-atrophy programs for astronauts need to be short in duration due to the time constraints imposed during spaceflight. Thus, we hypothesized that one set of ten repetitions of leg press, knee extension and seated calf raises, every other day, would be sufficient to ameliorate the loss of muscle cross-sectional area (CSA) and strength following the immobilization period as versus a control group. Since nutritional provision in close proximity to a resistance exercise stimulus appears to augment hypertrophy (Tipton *et al.*, 1999;Esmarck *et al.*, 2001;Cribb & Hayes, 2006;Andersen *et al.*, 2005), we also included a group that performed resistance exercise and immediately following consumed 30g of a whey protein supplement. They also consumed a second 30g whey protein supplement 6h hence (on their own recognizance) to again stimulate MPS; MPS is known to be responsive to nutrition for up to 8.5h following performance of exercise (Moore *et al.*, 2005). We proposed that the aforementioned exercise protocol in combination with a daily whey protein supplementation will result in full maintenance of muscle mass and strength following short-term immobilization as versus a control group.

CHAPTER II

LOW VOLUME RESISTANCE EXERCISE PREVENTS LOSS OF MUSCLE MASS AND FUNCTION DURING 14 DAYS OF KNEE IMMOBILIZATION

INTRODUCTION

The most conspicuous physiological adaptations to muscle disuse, namely skeletal muscle atrophy and a reduction in strength, have a rapid onset and are evident after only two weeks of unloading (Bamman *et al.*, 1998;Hespel *et al.*, 2001;Jones *et al.*, 2004;Yasuda *et al.*, 2005). Much of the research in non-disease mediated muscle atrophy has focused on describing the morphological and functional changes to skeletal muscle with unloading (Tesch *et al.*, 1994;Berg *et al.*, 1997;Hortobagyi *et al.*, 2000). Identifying countermeasures to atrophy that attenuate, if not completely prevent, loss of muscle mass and function during catabolic periods is important as even partial maintenance of muscle mass during situations of disuse may have a profound positive influence on rehabilitation (Hortobagyi *et al.*, 2000;Hespel *et al.*, 2001). Thus there is an increasing interest in alleviating atrophy and many studies have focused on effective countermeasures to atrophy, such as resistive exercise stimuli (Bamman *et al.*, 1997;Bamman *et al.*, 1998;Akima *et al.*, 2000;Akima *et al.*, 2001;Kawakami *et al.*, 2001;Akima *et al.*, 2003;Schulze *et al.*, 2002;Alkner & Tesch, 2004a;Tesch *et al.*, 2004;Mulder *et al.*, 2006) and nutritional intervention (Hespel *et al.*, 2001;Paddon-Jones *et al.*, 2005). Thus far, resistance exercise training paradigms, of workloads similar to those employed in eliciting hypertrophy, have been successful in preserving quadriceps femoris muscle mass and function during short-term (20-21d) unilateral lower limb suspension (ULLS) (Schulze *et al.*, 2002) and bed rest (BR) (Akima *et al.*, 2000;Kawakami *et al.*, 2001). Interestingly, a consistent finding in the literature is that despite successful maintenance of muscle mass and strength of the quadriceps femoris muscle group with a resistance exercise countermeasure, the triceps surae muscle group is considerably less responsive to the exercise stimulus and inevitably undergoes atrophy and/or strength loss (Akima *et al.*, 2000;Akima *et al.*, 2001;Alkner & Tesch, 2004a;Tesch *et al.*, 2004).

As its basis, atrophy arises due to a chronic negative protein balance or muscle protein breakdown exceeding synthesis. We have reported data, as have others, that net protein balance is greatest following a bout of resistance exercise with adequate amino acid availability (Rasmussen & Phillips, 2003;Phillips, 2004;Rennie *et al.*, 2004). Moreover, in the promotion of hypertrophy it appears that immediate post-exercise protein consumption both facilitates (Andersen *et al.*, 2005;Cribb & Hayes, 2006); Hartman *et al.*, In Press) and is, perhaps, necessary for hypertrophy to occur. Thus, as a potentially highly effective countermeasure to disuse atrophy, resistance exercise followed closely by an amino acid/protein supplementation may create an environment to optimally 'rescue' muscle atrophy resulting from disuse.

While atrophy countermeasure programs have employed resistance exercise in volumes similar to those used to invoke hypertrophy, we wished to see whether a much lower dose of resistance exercise than has been previously employed would be effective at alleviating atrophy. Thus, the purpose of this study was to evaluate the effectiveness of low volume, high-intensity resistance exercise, alone or in combination with a whey protein supplement, on the maintenance of muscle mass and strength during 14 days of knee-brace mediated immobilization in humans. We hypothesized that one set of ten repetitions of leg press (with plantar flexion at full extension), knee extension, and seated calf raises, every other day for a total of seven exercise sessions, would be sufficient to ameliorate the loss of muscle cross-sectional area (CSA) and strength normally observed following a 14 day immobilization period. Moreover, we propose that the aforementioned exercise protocol, in combination with a daily whey protein supplement, would result in full maintenance of muscle mass and strength following immobilization.

METHODS

Subjects

Seventeen recreationally active (i.e., exercise $\leq$ 2-3 $d \cdot wk^{-1}$) males and females volunteered to participate in the study. All subjects underwent 14 days of unilateral knee-brace mediated immobilization. Subjects were divided into the control group (CON), resistance exercise countermeasure group (EX), or the whey protein supplementation and resistance exercise countermeasure group (WHY+EX). Subjects were stratified into the groups to match for weight and height (Table 1). Subjects were screened to exclude smokers, any person with lower-limb injury within one year prior to the start of the study, or family history of thrombosis. The study was approved by the McMaster University and the Hamilton Health Sciences Research Ethics Boards and informed written consent was obtained from each participant prior to the commencement of the study.

Table 1: Subject anthropometric characteristics.

Group	Age (y)	Weight (kg)	Height (m)	BMI (kg·m^{-2})
CON (N = 3 ♀; N=2 ♂)	23.9 ± 2.2	73 ± 8	1.76 ± 0.06	23.4 ± 1.1
EX (N = 4 ♀; N=2 ♂)	22.5 ± 2.3	79 ± 8	1.72 ± 0.06	26.4 ± 1.6
WHY+EX (N = 5 ♀; N=1 ♂)	25.6 ± 2.0	76 ± 5	1.71 ± 0.03	26.1 ± 1.6

CON, knee-immobilization control group; EX, knee-immobilization with resistance exercise (RE) countermeasure (CM); WHY+EX – knee-immobilization with RE and whey protein supplement CM.

Experimental Protocol

All subjects were familiarized with the techniques to be used for muscle strength and function testing at least one week prior to beginning the study. These included isometric maximal voluntary contraction (MVC) for knee extension and plantar flexion and maximally evoked twitch contraction using muscle stimulation electrodes. Subjects in the EX and WHY+EX groups participated in an additional familiarization session with the strength training equipment (Universal Gym Equipment, West Point, MS). At this time, each subject's voluntary single-repetition maximum (1-RM) was determined for knee extension, leg press (with plantar flexion at full extension), and seated calf-raises exercises.

The study consisted of 14 days of unilateral knee immobilization. The knee chosen to be immobilized was done so in a randomized manner, counter-balanced for dominance based on strength in each leg. Testing was performed before immobilization (PRE; 1d prior to the start of 14d immobilization period) and after immobilization (POST; morning after 14d of immobilization). Measurements at each of these sessions included: thigh and lower leg CSA by magnetic resonance imaging (MRI); involuntary muscle twitch characteristics, percent motor unit activation (%MUA), and isometric MVC for knee extensor and plantar flexor muscle groups using custom-built dynamometers, previously utilized by Hamada and colleagues (Hamada *et al.*, 2003). The MRI was conducted on the afternoon prior to the PRE testing session since subjects were immobilized on the morning on day 1.

On the first day of immobilization, subjects arrived in the lab by 0900 hrs and were fitted with a knee immobilization brace (GII Rehab Contour Air Light, Orthotics Inc., Ossur, ON, Canada) and a set of crutches. Adhesive tape was applied over the Velcro® strap of the brace and the investigator's signature was affixed over the seal to ensure the integrity of the brace and compliance with the immobilization procedures. Subjects returned to the lab daily, at which time they were permitted to remove the brace, under supervision, for approximately 15 minutes to allow for inspection by the investigators. Visual inspection of the immobilized leg and knee brace were performed, and any signs of chaffing or swelling were noted and the brace readjusted. Following visual inspection the brace was reapplied and secured as described above. All subjects found the brace tolerable, did not report any adverse events such as tightness swelling, and only minor chaffing and itching which was usually correctable by adjusting some aspect of the brace.

Every other day, when participants reported to the lab, during the 15 minute visual inspection period when the knee brace was removed, subjects in the EX and WHY+EX groups performed a bout of resistance exercise training. The exercise consisted of one set of 10±2 repetitions at 80% 1-RM, every other day, for knee extension, leg press with plantar flexion at full extension, and seated calf raises. The total volume of resistance exercise during the training was 30 contractions every 48 hours.

The CON and EX groups and the WHY+EX group consumed two 250mL boluses, daily, of either an isocaloric carbohydrate beverage or a whey protein supplement, respectively (Table 2). All three groups consumed the first 250ml bolus under supervision while in the lab for their daily visit. Subjects were instructed to consume the second 250mL bolus mid-afternoon between meals. On the RE training days, the EX and WHY+EX groups consumed the first bolus immediately post-exercise (under supervision) and the second bolus one hour later. Cocoa was added to the drinks to increase palatability and to blind the drinks' taste; maltodextrin was used in the carbohydrate beverage. A detailed profile of the amino acid composition of the whey protein isolate (Protient 9500, Protient Inc., St. Paul, MN) is attached in Appendix 7.

Table 2: Composition of whey supplement and carbohydrate beverage (per 250 ml)

Ingredient	Whey Protein Isolate Beverage	Carbohydrate Beverage
Whey Protein Isolate (PROTIENT 9500)	30 g	0 g
Sucralose (Splenda®)	2 g	0 g
Cocoa	2 g	2 g
Maltodextrine	0 g	20 g
Granulated Sugar	0 g	10 g
Total Energy	**128 kcal**	**128 kcal**

Diet Records

Subjects completed 3-day diet records during the week prior to the immobilization period (DR1) and during days 8-14 of the immobilization period (DR2). These dietary records were analyzed (Nutritionist V, First Data Bank) for distribution of macronutrient consumption, average total calories per day, and protein intake.

Magnetic Resonance Imaging (MRI)

The MRI was performed in a 3-Tesla HD scanner (Signa MRI system, GE Medical, Wilwaukee, WI) at the Brain-Body Institute, Imaging Research Centre, St. Joseph's Healthcare (Hamilton, ON). Image acquisition was carried out using a T1 flair in the axial plane with the following parameters: repetition time/echo time = 2574ms/6.7ms; field of view = 25-30cm; matrix size = range from 320/320 to 512/512 phase/freq; inversion time = 958ms; slice thickness = 5mm. Thigh image acquisition utilized an 8-channel torso coil with 2 number of excitations (NEX) and the calf image was collected using a single channel transmit/receive extremity coil with 4 NEX. During the PRE scan, bony landmarks for the thigh and lower leg (specifically, superior greater trochanter of the femur and medial tibial plateau of the tibia) were identified and the distance from those landmarks to the first axial scan was recorded. These distances were used in the POST scan to ensure identical positioning for the thigh and lower leg scans. A total of nine slices were obtained from the mid-thigh and seven slices from the mid-lower leg. The MRI images were transferred to a personal PC and analyses were performed using Medical Image Processing, Analysis and Visualization (MIPAV) software (downloaded with permission from the National Institutes of Health;

http://mipav.cit.nih.gov/). A bilateral scan was performed on a subset of five subjects to calculate changes in CSA for the contra-lateral (non-immobilized) leg.

Muscle Strength

For isometric knee extension (KE) strength testing, subjects were seated on a bench with their immobilized leg secured in a custom-made dynamometer equipped with a strain gauge previously utilized by Hamada and colleagues (Hamada *et al.*, 2003). For the knee extension strength testing, the subject was seated upright, positioned with their trunk at 90° to horizontal and their knee joint was fixed at an angle of 110° (70° below horizontal) and securely fastened with Velcro® straps. Maximal isometric strength was taken as the peak torque achieved during a 5 second MVC. The MVC was repeated 3 times with a minimum of 2 minutes rest between trials. Similarly, for isometric plantar flexion (PF) strength testing, subjects were seated in a chair with the lower segment of their immobilized leg secured in a custom-made dynamometer. Subject was seated upright with their trunk 90° to horizontal, their knee joint was bent to 90° and the ankle joint was 10° in dorsiflexion (10° above horizontal). The same protocol was followed to determine the isometric strength of the plantar flexion muscle group as described for KE strength.

Involuntary Muscle Function

Muscle twitch characteristics were measured using a method previously described by Hamada and colleagues (Hamada *et al.*, 2003). Briefly, a percutaneous electrical impulse was used to stimulate the femoral nerve and the medial popliteal nerve to elicit an involuntary twitch in the knee extensors and plantar flexors muscle groups, respectively. Prior to applying the stimulation electrodes, electrode gel was applied to the surface electrode and the underlying skin. The stimuli were rectangular voltage pulses, 200μs in duration derived from a stimulator (Devices 3072, Medical Systems, Welwyn, Garden City, Herts, UK). The evoked maximal peak twitch torque (PTT) was obtained by administering a series of 200μs pulses of increasing voltage until an increase in voltage of ~20% elicited no further increase in PTT. Subjects were required to rest for 15 minutes prior to collection of any resting twitch data. A total of three resting twitches were obtained and were analyzed for rise time (Rt), PTT, and half relaxation time (HRt).

The %MUA was determined using an adaptation of the method previously described by Kawakami and colleagues (Kawakami *et al.*, 2001). Briefly, a supra-maximal electrical stimulus was externally applied to a muscle during a maximal voluntary isometric contraction. The resulting interpolated twitch torque (ITT) is expressed relative to the evoked resting peak twitch torque (PTT) and %MUA calculated using the equation: %MUA = (1-(ITT/PTT)) x 100 %.

The M-wave associated with the evoked twitches and the surface electromyography (EMG) associated with the MVC were recorded from the vastus medialis and soleus muscles for knee extension and plantar flexion, respectively. Ag/AgCl electromyographic (EMG) disposable recording electrodes (3.8 mm diameter) were applied to the surface of the skin above the vastus medialis and soleus muscles to record electrical data from the muscle. EMG signals were amplified (1000x) and filtered (10 Hz-2 kHz) as previously described by Hamada and colleagues (Hamada *et al.*, 2003).

Statistical Analyses

A two-factor analysis of variance (ANOVA) with repeated measures within time (PRE and POST immobilization) and between group (CON, EX, WHY+EX) was utilized for all statistical analyses. Tukey post-hoc tests were employed to make pair-wise comparisons following identification of significance by ANOVA. Analyses were conducted using computer statistical software program (Sigma Stat, v. 3.1, Systat Software, Inc, San Jose, CA). Statistical differences were accepted at $p<0.05$. All data are presented as means ± SEM.

RESULTS

Thigh and lower leg muscle cross-sectional area

There was a 6.2±1.7% reduction in the muscle CSA of the thigh for the CON group following 14 days of knee immobilization, with no change over time in the EX or WHY+EX groups ($p<0.05$; Figure 1). There was a significant reduction in CSA of the leg for all three groups ($p<0.05$; Figure 2), with a trend for greater losses in the CON group ($p=0.065$; Figure 2).

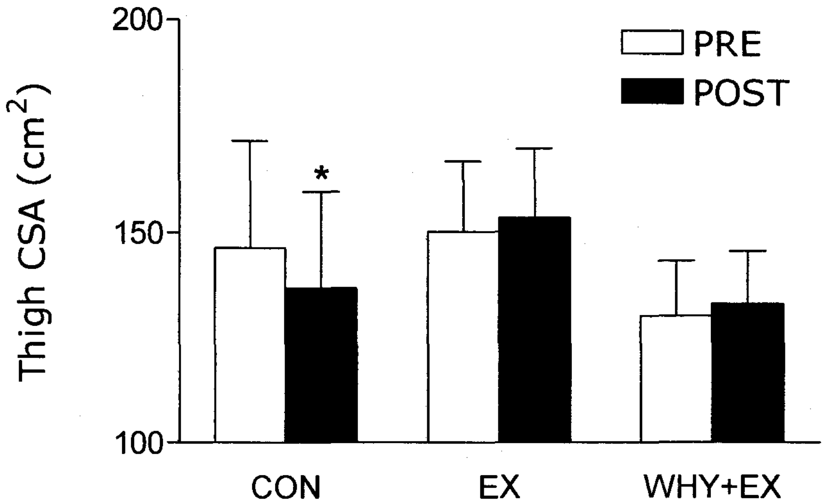

Figure 1: MRI muscle cross-sectional areas (cm^2) for the thigh before and after 14 days of unilateral knee immobilization. * significantly different from PRE, $p<0.05$. Values are means ± SEM.

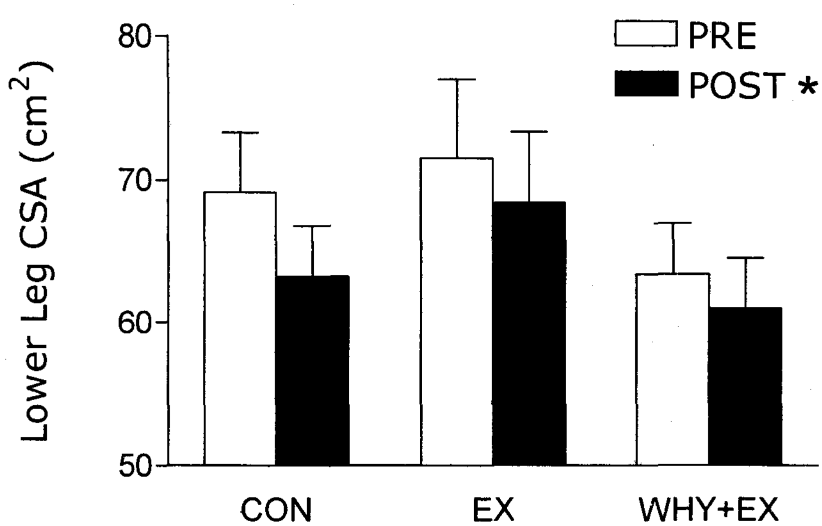

Figure 2: MRI muscle cross sectional area (cm^2) of the lower leg before and after 14 days of unilateral knee immobilization. * significant main effect for time. Values are means ± SEM.

Individual whole muscle cross-sectional area

The CON group showed a 7.6±3.2% reduction for the quadriceps femoris ($p<0.05$; Table 3). Similar to total thigh CSA, there were no changes in quadriceps femoris CSA following the 14 day immobilization period for either of the EX or WHY+EX groups ($p<0.05$). With respect to the individual muscles of the lower leg, the gastrocnemius showed significant reductions for all three groups post-immobilization ($p<0.05$; Table 3). Conversely, the soleus showed a 6.8±2.1% reduction for the CON group ($p<0.05$; Table 3), with no changes in CSA following immobilization for either of the EX or WHY+EX groups.

Table 3: Summary of muscle cross-sectional areas (CSA) for quadriceps femoris, triceps surae, gastrocnemius and soleus muscle.

		CSA (cm^2)		
Muscle	**Group**	**PRE**	**POST**	**% Change**
Quadriceps Femoris	CON	67.2 ± 12.8	61.1 ± 10.4 *	-7.6 ± 3.2
	EX	69.8 ± 7.0	72.5 ± 7.4	3.8 ± 0.6
	WHY+EX	59.4 ± 5.6	60.4 ± 5.5	1.8 ± 2.5
Triceps Surae	CON	39.9 ± 2.0	36.6 ± 1.6	-8.1 ± 2.2
	EX	39.2 ± 5.4	37.4 ± 5.1	-4.2 ± 1.0
	WHY+EX	37.6 ± 2.5	36.1 ± 2.7	-4.3 ± 0.8
Gastrocnemius	CON	19.9 ± 0.9	18.0 ± 0.6	-9.4 ± 2.4
	EX	22.1 ± 2.9	20.9 ± 2.4	-6.0 ± 1.4
	WHY+EX	20.5 ± 1.1	19.0 ± 1.0	-7.2 ± 1.2
Soleus	CON	20.0 ± 1.2	18.6 ± 1.1 *	-6.8 ± 2.1
	EX	19.8 ± 2.0	19.3 ± 1.9	-2.1 ± 1.2
	WHY+EX	17.4 ± 1.6	17.4 ± 1.9	-0.5 ± 2.0

* significantly different from PRE. Values are means ± SEM.

The CSA of quadriceps femoris, soleus, and gastrocnemius was quantified from images obtained from the contralateral (non-immobilized) leg. There were no changes in CSA in the non-immobilized leg (N=5) following 14 days of unilateral knee immobilization for quadriceps femoris (0.4±1.3%, p=0.63), soleus (-0.3±1.8%, p=0.91), and gastrocnemius (4.5±2.9%, p=0.15).

Isometric maximal voluntary contraction (MVC)

With respect to knee extension strength, there was a 22.3±4.0% reduction in isometric torque production for the CON group following the immobilization period ($p<0.05$; Figure 3). There were no changes in knee extension MVC for both the EX and WHY+EX groups. Plantar flexion strength exhibited similar loses of approximately 25.3±2.5% in the CON group ($p<0.05$; Figure 4), and likewise no reduction in the EX and WHY+EX groups.

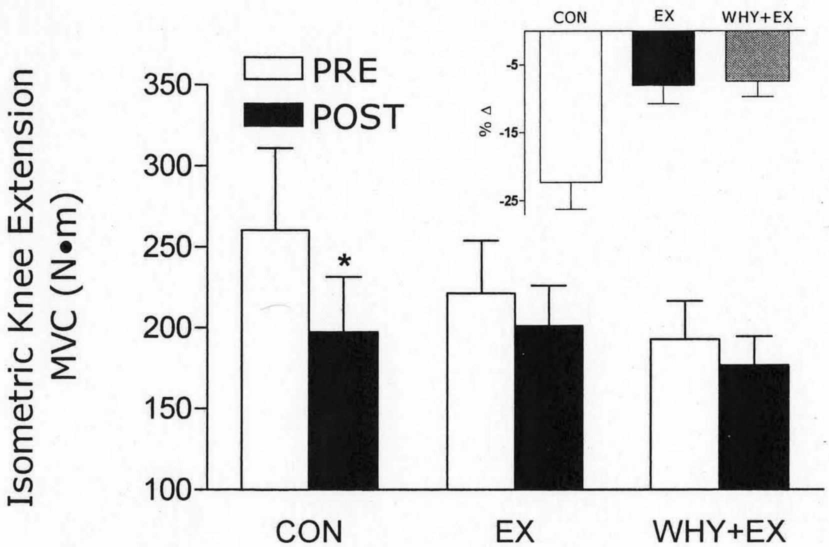

Figure 3: Knee extension MVC (N·m) before and after 14 days of unilateral knee immobilization. * significantly different from PRE. Inset: % change from PRE. Values are means ± SEM.

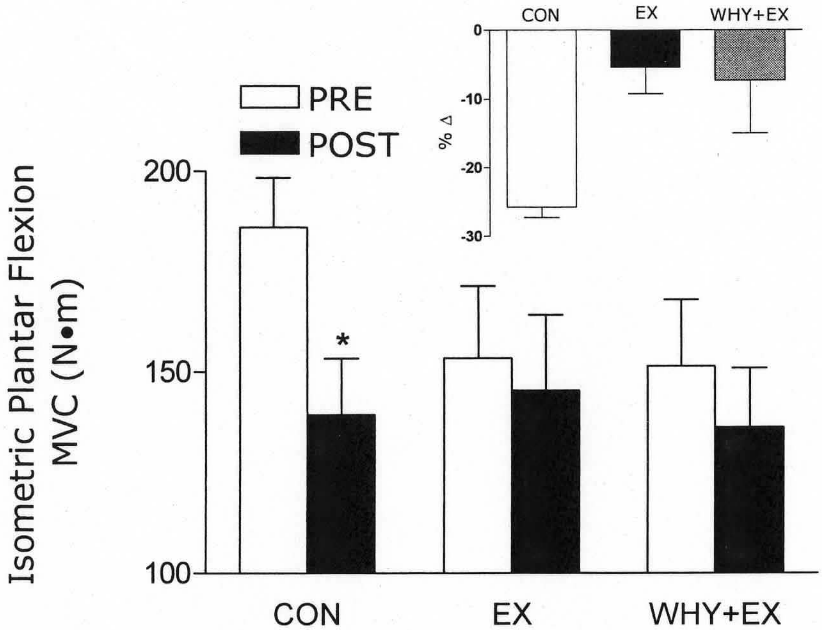

Figure 4: Plantar flexion MVC (N·m) before and after 14 days of unilateral knee immobilization. * significantly different from PRE. Inset: % change from PRE. Values are means ± SEM.

Specific Strength

There was a significant reduction in knee extension and plantar flexion specific strength post-immobilization for all three groups ($p<0.05$) with no between group differences (Figure 5 and 6). For plantar flexion specific strength, the CON group tended towards greater losses in specific strength than EX and WHY+EX ($p=0.068$).

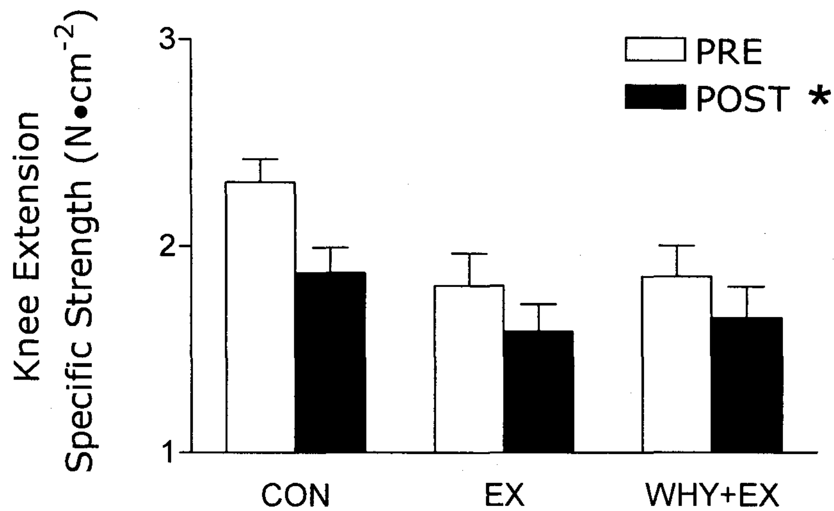

Figure 5: Knee extension specific strength before (PRE) and following (POST) 14 days of unilateral knee immobilization. * significant main effect for time. Values are means ± SEM.

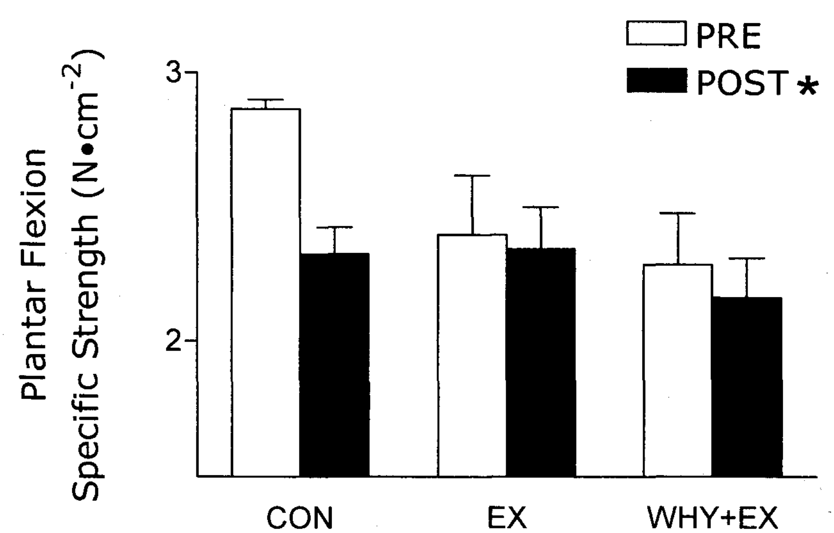

Figure 6: Plantar flexion specific strength before (PRE) and following (POST) 14 days of unilateral knee immobilization. * significant main effect for time. Values are means ± SEM.

Percent Motor Unit Activation (%MUA)

Motor unit activation for both knee extension and plantar flexion was unaltered following 14 days of unilateral knee immobilization (Table 4).

Table 4: Knee extension and plantar flexion %MUA values before (PRE) and immediately following (POST) 14 days of unilateral knee immobilization.

Percent Motor Unit Activation (%MUA)				
	Knee Extension		Plantar Flexion	
Group	PRE	POST	PRE	POST
CON	94.6 ± 2.0	98.2 ± 0.8	95.4 ± 1.0	95.7 ± 1.5
EX	92.3 ± 3.5	91.1 ± 2.4	94.4 ± 2.2	93.9 ± 2.1
WHY+EX	93.2 ± 2.0	92.5 ± 2.4	94.3 ± 1.7	96.8 ± 0.9

Rise Time (Rt), Peak Twitch Torque (PTT), and Half Relaxation Time (HRt)

The knee extension maximally evoked twitch showed significantly longer Rt post-immobilization in the CON group ($p<0.05$; Table 5). Conversely, the EX group showed no change while the WHY+EX had a significant reduction in Rt post-immobilization ($p<0.05$; Table 5). Plantar flexion twitch Rt was significantly longer in the CON and EX groups, with no change in the WHY+EX group ($p < 0.05$; Table 6).

Table 5: Knee extension single twitch contraction characteristics.

		Rt (ms)	% Δ	PTT (N·m)	% Δ	HRt (ms)	% Δ
CON	PRE	55.7 ± 2.5	5.2 ± 2.3	39.1 ± 7.9	-13.9 ± 7.3	66.8 ± 2.7	-7.8 ± 4.3
	POST	58.5 ± 1.9 *		32.1 ± 4.3		61.3 ± 2.2	
EX	PRE	49.3 ± 3.5	3.9 ± 1.4	37.4 ± 6.7	5.1 ± 7.9	61.9 ± 3.6	8.4 ± 4.0
	POST	51.2 ± 3.6		37.5 ± 4.9		66.4 ± 2.4 *	
WHY+EX	PRE	51.4 ± 1.2	-6.3 ± 1.5	32.9 ± 4.0	10.7 ± 8.6	66.3 ± 2.9	9.3 ± 4.5
	POST	48.2 ± 1.2 *		35.7 ± 3.9		71.9 ± 1.6 *	

* significantly different from PRE. Rt, rise time; PTT, peak twitch torque; HRt, half relaxation time. All values means ± SEM.

Table 6: Plantar flexion single twitch contraction characteristics.

		Rt (ms)	% Δ	PTT (N·m)	% Δ	HRt (ms)	% Δ
CON	PRE	68.9 ± 3.7	18.4 ± 4.5	23.1 ± 2.9	-3.7 ± 7.0	100.7 ± 2.8	25.3 ± 7.4
	POST	81.0 ± 4.2 *		21.6 ± 1.5		126.5 ± 9.7 †	
EX	PRE	62.2 ± 3.4	10.1 ± 2.4	25.0 ± 1.7	0.3 ± 1.5	106.5 ± 4.0	14.3 ± 5.3
	POST	68.4 ± 3.8 *		25.0 ± 1.5		121.4 ± 5.9 †	
WHY+EX	PRE	75.4 ± 4.9	1.6 ± 4.4	20.5 ± 1.7	4.3 ± 5.0	116.4 ± 4.0	0.8 ± 6.0
	POST	76.1 ± 4.7		21.3 ± 2.0		117.0 ± 7.0 †	

* significantly different from PRE. † significant main effect of time. Rt, rise time; PTT, peak twitch torque; HRt, half relaxation time. All values means ± SEM.

PTT showed a trend toward greater losses in the CON group for knee extension ($p=0.06$) as compared to the combined EX and WHY+EX groups. EX and WHY+EX had significantly longer ($p<0.05$; Table 5) knee extension twitch HRt, with no changes observed for the CON. Plantar flexion twitch HRt was significantly longer for all three groups ($p<0.05$; Table 6) with a trend towards greater increases in the CON group ($p = 0.065$).

Dietary Analysis

Two three-day dietary records were collected: the first was collected during the week prior to the immobilization period (DR1) and the second was collected during days 8-14 of immobilization (DR2). There were no significant differences in caloric intake for any of the three groups (Table 7), as well as no differences in the percentage of kcal derived from each of carbohydrate, protein and fat when comparing the three groups across the two three-day diet records (Table 8). The protein intake for the WHY+EX group was not different between DR1 (1.04±0.13 g/kg/d) and DR2 (1.25±0.19 g/kg/d) ($p=0.17$).

Table 7: Average caloric intake before (DR1) and during days 8-14 (DR2) of the 14 day immobilization period.

Average Kilocalories per day ($kcal \cdot d^{-1}$)		
Group	**DR1**	**DR2**
CON	1914 ± 370	1856 ± 134
EX	2074 ± 295	1947 ± 282
WHY+EX	1820 ± 179	2187 ± 253

Table 8: Percentage of total energy derived from carbohydrate, protein and fat before and during days 8-14 of the 14 day immobilization period.

% Total energy from carbohydrate (CHO), protein (PRO) and fat (FAT)			
Group	**CHO**	**PRO**	**FAT**
CON-Pre	55.4 ± 4.4	15.8 ± 1.9	30.0 ± 2.4
CON-Post	60.0 ± 3.4	15.6 ± 1.6	26.4 ± 2.6
EX-Pre	54.8 ± 1.8	15.0 ± 1.0	32.0 ± 2.5
EX-Post	56.8 ± 2.3	16.0 ± 1.1	28.8 ±1.4
WHY-EX-Pre	55.0 ± 2.8	15.5 ± 1.4	28.8 ± 1.8
WHY-EX-Post	54.0 ± 1.3	16.5 ± 1.8	26.7 ± 2.2

DISCUSSION

The novel finding of the present study is that a relatively low volume of high-intensity resistance exercise was an effective countermeasure against muscle mass and strength loss observed during 14 days of knee immobilization. Indeed, performance of only 30 contractions every other day at 80% 1-RM completely prevented losses in quadriceps extension strength and muscle CSA and in plantar flexion strength. We did not observe, as we had hypothesized, that the early provision of a protein supplement following resistance exercise was any more effective than resistance exercise alone. The extent of atrophy observed in the CON group was comparable with data published from other short term (2-3 weeks) disuse studies for quadriceps femoris muscle group (~7%) (Adams *et al.*, 1994;Akima *et al.*, 2000;Akima *et al.*, 2001;Hespel *et al.*, 2001;Schulze *et al.*, 2002;Jones *et al.*, 2004;Yasuda *et al.*, 2005) and the triceps surae muscle group (~8%) (Schulze *et al.*, 2002), suggesting that our model of disuse atrophy was appropriate.

Several studies have successfully ameliorated the unloading-induced muscle mass and strength losses with the use of resistance exercise countermeasures (Bamman *et al.*, 1998;Akima *et al.*, 2000;Kawakami *et al.*, 2001;Alkner & Tesch, 2004a;Tesch *et al.*, 2004). However, only one previous study has investigated whether a low dose of high-intensity resistance exercise would protect against losses in muscle size and function (Schulze *et al.*, 2002). Schulze and colleagues employed 3 sets (1 at 40% 1-RM, 2 at 80-85% 1-RM) in addition to 2-5 second isometric MVC for knee extension and plantar flexion, with exercises performed every third day. The present study, after correcting for frequency of resistance exercise training sessions, performed approximately 65-70% of the number of repetitions used previously (Schulze *et al.*, 2002). Thus, in an effort to define the minimal dose necessary to maintain mass and function of skeletal muscle we report here that one set of leg press (with plantar flexion at full knee extension), knee extension, and seated calf raises, every other day, is effective at maintaining total thigh, quadriceps femoris, and soleus muscle CSA. This exercise stimulus, however, did not completely preserve the lower leg, or specifically, the gastrocnemius/triceps surae muscle CSA. Our data suggests that the triceps surae muscle group is less responsive to the resistance exercise countermeasure than the quadriceps femoris muscle following unloading which is in agreement with previous studies (Akima *et al.*, 2000;Akima *et al.*, 2001;Alkner & Tesch, 2004a;Alkner & Tesch, 2004b;Tesch *et al.*, 2004).

It is likely a type II statistical error ($p=0.065$ for interaction) that we did not observe a preservation of muscle CSA in the lower leg muscle group in the EX and WHY+EX groups; however, our data are at least suggestive that the countermeasure was somewhat effective at attenuating the inactivity-induced atrophy in the calf. Indeed, in the CON group the losses in the gastrocnemius and soleus muscles, the two constituent muscles of the triceps surae, were 9.0±2.4% and 6.8±2.1% respectively. Interestingly, the gastrocnemius muscle showed an almost uniform reduction in CSA across the three groups whereas the soleus muscle showed full maintenance of CSA following the immobilization period for both the EX and WHY+EX groups. Thus, it appears that the lack of maintenance of CSA in the triceps surae muscle group following immobilization is a consequence of inadequate preservation of the gastrocnemius muscle, which overwhelmed the effective maintenance of CSA seen in the soleus muscle. This result is not too surprising when you consider the muscle activity during the resistance exercise training, specifically the almost exclusive soleus activity during the seated calf raises. While the present work implies additional stimulation may be required for full maintenance of muscle mass in the lower leg, it also provides evidence of a responsiveness of the postural soleus muscle to a low volume of high-intensity resistance exercise countermeasure. Thus, our data highlights the importance of examining each muscle individually and not simply an aggregate measure of the triceps surae or lower leg.

Our exercise paradigm was effective at mitigating the losses in MVC resulting in non-significant reductions of 9% for knee extension (KE) and 6% in plantar flexion (PF), with no differences between EX and WHY+EX treatment groups. The decrement in knee extension MVC torque production observed in the CON (-22.3±4.0%) was comparable to other short-term (2-3 weeks) disuse studies for KE (Hespel *et al.*, 2001;Deschenes *et al.*, 2002;Jones *et al.*, 2004). However, plantar flexion MVC torque decrement (-25.3±2.5%) was slightly greater than values previously reported (Schulze *et al.*, 2002). The utilization of the knee immobilization brace, at a fixed position of 90 degrees, could in part explain greater losses in plantar flexion strength versus data reported for unilateral lower limb suspension. The fixed joint angle resulted in the lower leg being completely non-weight bearing for the entire immobilization period without the possibility of even brief periods of weight bearing.

Despite maintenance of KE and PF MVC with the countermeasures, there was a significant reduction in specific strength (strength per unit muscle area) for all three experimental groups across both muscle groups. This result suggests a disproportionate strength loss relative to the degree of atrophy. Interestingly, the %MUA was unaltered following the immobilization period for all three groups. Thus, the ability of the central nervous system to recruit all motor units at maximal firing frequencies (i.e., %MUA) for the knee extensor and plantar flexor muscle groups was not compromised following 14 days of unilateral knee immobilization. Moreover, the maximal integrated surface electromyography (iEMG) recordings from the vastus medialis muscle and soleus muscle, measured during isometric MVC, were unaltered for all three groups following the immobilization period. Taken together, these results suggest the reduction in specific strength ($N \cdot cm^{-2}$) observed was not mediated through an adaptation in the central nervous system but rather through a mechanism intrinsic in the muscle.

We observed a trend ($p = 0.06$) towards a greater reduction in PTT for the CON group versus the combined countermeasure groups (EX, and WHY+EX) in the knee extensor involuntary twitch. The corresponding electrical activity of the muscle (as measured by maximal iEMG), however, was unaltered post-immobilization during an MVC, suggesting an inefficiency to translate the muscle electrical activity into muscle torque production. With regards to a single twitch profile, the rise time (Rt) was also significantly longer post-immobilization in the CON group for both KE and PF suggesting a dysfunction in calcium release from the sarcoplasmic reticulum (SR).

When we consider that central neural drive was not compromised following 14 days of immobilization, the observed alterations in the twitch profile characteristics can be explained, in part, by the phenomenon of low frequency fatigue (LFF) first described by Edwards and colleagues (Edwards *et al.*, 1977). LFF is characterized by a reduction in torque generating capacity of skeletal muscle without alterations in electrical activity in the muscle and in the absence of metabolic by-products (Jones, 1996); each of these conditions are consistent with the present study. By applying the theory of LFF and its proposed mechanism of dysfunction, namely impairment in excitation-contraction (EC) coupling, we can speculate that alterations in twitch characteristics and observed reductions in specific strength may be associated with alterations in ionic Ca^{2+} release and re-uptake from the SR (Jones, 1996). Clearly, further studies are required to identify the precise mechanism(s) underlying the observed changes. We propose measuring the expression of key proteins involved in Ca^{2+} release (dihydropyridine and ryanodine receptors) and re-uptake from the SR (sarco/endoplasmic reticulum Ca^{2+} ATPase, SERCA) following immobilization.

It is known that provision of amino acids in the post-exercise period is important for maximizing protein synthesis and muscle mass accrural (Tipton *et al.*, 1999;Esmarck *et al.*, 2001;Cribb & Hayes, 2006;Andersen *et al.*, 2005); Hartman *et al.*, In Press; Tang *et al.*, In Press) To our knowledge, this is the first study to investigate the potential additive effect of post-exercise protein supplementation, during an immobilization period, on the maintenance of muscle mass and strength. Our results suggest that there was no additional benefit to twice daily whey protein supplementation in maintaining muscle mass or strength. There are two possible explanations for this result. First, it is likely that the habitual daily protein consumption of the subjects was sufficient to maximize the post-exercise response; hence the whey supplements were of no additional benefit. Secondly, despite efforts to provide a supplement with an optimal dose of EAA (Cuthbertson *et al.*, 2005) with particular attention to the timing of the consumption, the subjects in the WHY+EX group voluntarily reduced their habitual intake of protein such that the whey protein drinks were not truly a protein supplement but rather a protein replacement (i.e., daily protein consumption did not change significantly ($p = 0.17$)). Although the diets of the subjects were not controlled, the subjects were encouraged to maintain their normal dietary habits. However, since subjects in the EX and WHY+EX groups were blinded to whether they were consuming the whey supplement or the isocaloric carbohydrate beverage, it is possible that the high level of satiety associated with two 30g doses of whey protein each day may have resulted in an inadvertent reduction in habitual protein/energy intake, which is consistent with our findings (Anderson *et al.*, 2004). It may also be that the benefits of such a whey protein supplement are only seen with longer periods of immobilization or when subjects are in a hypoenergetic state.

In conclusion, the present study provides evidence that inactivity-induced muscle mass and strength losses can be prevented with low volume, high-intensity resistance training during the disuse period. The successful development of such resistance exercise and nutritional countermeasures will have implications for persons exposed to bed rest, long duration spaceflight missions, and more widely, to any persons experiencing loss of muscle mass due to inactivity. Our results are in agreement with others who have suggested that the lower leg is less responsive to resistance exercise countermeasures, thus more susceptible to wasting, than the thigh during immobilization, however the resistance exercise training paradigm we utilized was effective at preserving the postural soleus muscle. Furthermore, our data suggests that there is no additional benefit to daily whey protein supplementation during an immobilization period in the maintenance of muscle mass or function, however, further studies with complete control over dietary intake would needed to confirm this finding.

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APPENDIX 1

SUBJECT ANTHROPOMETRIC CHARACTERISTICS

Subject Anthropometric Characteristics

Subject	Group	Weight (kg)	Height (cm)	Leg Immob	Age (yr)	Gender	BMI (kg/m^2)
S5	CON	56	163	R	24.8	F	21.1
S8	CON	73	180	L	19.2	F	22.5
S9	CON	91	184	R	25.2	M	26.9
S12	CON	92	192	L	19	M	25.0
S18	CON	54	159	L	30.7	F	21.4
	MEAN	73	176		23.8		23.4
	SEM	8	6		2.2		1.1
S4	EX	59	170	L	20	F	20.4
S6	EX	100	177	L	18.8	M	31.9
S11	EX	75	161	L	24	F	28.9
S13	EX	104.9	198	R	33.1	M	26.8
S15	EX	68	166	R	19.7	F	24.7
S16	EX	67.8	162	L	19.1	F	25.8
	MEAN	79	172		22.5		26.4
	SEM	8	6		2.3		1.6
S1	WHY+EX	59	168	L	23.8	F	20.9
S7	WHY+EX	77	183	R	21.7	M	23.0
S10	WHY+EX	93.8	172	R	31.3	F	31.7
S14	WHY+EX	76	174	L	30	F	25.1
S17	WHY+EX	74	164	R	19	F	27.5
S19	WHY+EX	75.8	163	R	28	F	28.5
	MEAN	76	171		25.6		26.1
	SEM	5	3		2.0		1.6

APPENDIX 2

MUSCLE CROSS-SECTIONAL AREA

& ANOVA TABLES

MRI CSA Area Raw Data

Subject	Group	Time	Thigh	QF	VL	Lower Leg	TS	Sol	Gast
S5	CON	PRE	92.00	43.04	19.20				
S5	CON	POST	92.26	43.15	18.75				
S8	CON	PRE	124.22	49.28	20.35	64.68	37.27	19.50	17.76
S8	CON	POST	113.41	49.07	20.09	57.94	36.14	19.04	17.10
S9	CON	PRE	218.06	106.74	39.32	78.09	41.43	20.05	21.38
S9	CON	POST	200.67	91.92	33.43	71.13	35.70	17.60	18.10
S12	CON	PRE	194.15	88.76	38.95	73.91	44.63	23.11	21.52
S12	CON	POST	182.96	80.33	34.39	67.29	41.10	21.40	19.70
S18	CON	PRE	103.34	48.16	19.24	59.77	36.15	17.21	18.95
S18	CON	POST	94.30	41.09	15.35	56.60	33.36	16.28	17.08
	PRE	**MEAN**	**146.4**	**67.2**	**27.4**	**69.1**	**39.9**	**20.0**	**19.9**
		SEM	**25.2**	**12.8**	**4.8**	**4.2**	**2.0**	**1.2**	**0.9**
	POST	**MEAN**	**136.7**	**61.1**	**24.4**	**63.2**	**36.6**	**18.6**	**18.0**
		SEM	**23.0**	**10.5**	**4.0**	**3.5**	**1.6**	**1.1**	**0.6**
S4	EX	PRE	135.05	56.22	20.63	57.03	34.50	14.97	19.53
S4	EX	POST	135.41	57.78	21.72	56.80	33.81	15.41	18.40
S6	EX	PRE	186.60	95.45	39.22	92.15	57.53	22.51	35.03
S6	EX	POST	189.94	99.07	39.97	86.41	52.89	21.57	31.33
S11	EX	PRE	111.89	55.45	23.25	60.86	30.83	16.68	14.15
S11	EX	POST	115.62	56.26	22.95	61.34	29.90	16.52	13.38
S13	EX	PRE	214.15	87.45	38.61	83.01	50.38	28.31	22.07
S13	EX	POST	215.30	91.56	40.66	80.72	49.52	27.69	21.83
S15	EX	PRE	126.11	60.98	60.98	68.93	21.19	18.68	18.68
S15	EX	POST	134.59	63.97	63.97	63.39	19.20	18.15	18.15
S16	EX	PRE	127.61	63.19	26.09	67.12	40.64	17.61	22.99
S16	EX	POST	130.40	66.36	27.68	61.74	38.85	16.71	22.12
	PRE	**MEAN**	**150.2**	**69.8**	**34.8**	**71.5**	**39.2**	**19.8**	**22.1**
		SEM	**16.5**	**7.0**	**6.1**	**5.5**	**5.4**	**2.0**	**2.9**
	POST	**MEAN**	**153.5**	**72.5**	**36.2**	**68.4**	**37.4**	**19.3**	**20.9**
		SEM	**16.1**	**7.4**	**6.5**	**4.9**	**5.1**	**1.9**	**2.5**
S1	WHY+EX	PRE	114.50	50.75	17.96	58.05	35.78	16.36	19.42
S1	WHY+EX	POST	112.70	47.45	16.37	57.11	33.90	15.84	18.06
S7	WHY+EX	PRE	193.47	86.15	27.26	76.99	47.81	23.68	24.13
S7	WHY+EX	POST	192.62	85.05	26.02	75.83	47.26	25.42	21.84
S10	WHY+EX	PRE	131.50	61.34	22.65	69.72	34.65	14.73	22.02
S10	WHY+EX	POST	136.72	62.04	21.58	66.04	33.48	14.53	21.03
S14	WHY+EX	PRE	115.93	55.66	21.00	64.12	42.48	20.59	21.89
S14	WHY+EX	POST	123.04	58.87	22.13	59.95	40.78	20.41	20.37
S17	WHY+EX	PRE	111.65	53.44	17.75	57.82	33.67	15.54	18.14
S17	WHY+EX	POST	119.87	59.43	19.72	55.22	31.86	15.83	16.03
S19	WHY+EX	PRE	114.2771	49.06	19.84	53.70	31.38	13.70	17.13
S19	WHY+EX	POST	113.9395	49.41	19.93	51.74	29.29	12.77	16.41
	PRE	**MEAN**	**130.2**	**59.4**	**21.1**	**63.4**	**37.6**	**17.4**	**20.5**
		SEM	**13.0**	**5.6**	**1.4**	**3.6**	**2.5**	**1.6**	**1.1**
	POST	**MEAN**	**133.1**	**60.4**	**21.0**	**61.0**	**36.1**	**17.5**	**19.0**
		SEM	**12.4**	**5.5**	**1.3**	**3.6**	**2.7**	**1.9**	**1.0**

Thigh

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Group	2	24500768.267	12250384.133	0.352	0.710
Subject(Group)	14	487875465.898	34848247.564		
Time	1	108438.216	108438.216	1.009	0.332
Group x Time	2	2872543.003	1436271.502	13.370	<0.001
Residual	14	1503897.715	107421.265		
Total	33	516786790.407	15660205.770		

Comparisons for factor: **Time within CON**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
PRE vs. POST	963.595	2	6.574	<0.001	Yes

Quadriceps Femoris Muscle

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Group	2	7734829.384	3867414.692	0.522	0.604
Subject(Group)	14	103678725.699	7405623.264		
Time	1	53763.709	53763.709	0.694	0.419
Group x Time	2	1154834.568	577417.284	7.455	0.006
Residual	14	1084388.211	77456.301		
Total	33	113672958.985	3444635.121		

Comparisons for factor: **Time within CON**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
PRE vs. POST	608.499	2	4.889	0.004	Yes

Vastus Lateralis Muscle

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Group	2	12934262.061	6467131.030	2.779	0.096
Subject(Group)	14	32580757.311	2327196.951		
Time	1	29305.099	29305.099	1.940	0.185
Group x Time	2	265349.617	132674.809	8.781	0.003
Residual	14	211529.278	15109.234		
Total	33	46008879.667	1394208.475		

Comparisons for factor: **Time within CON**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
PRE vs. POST	300.962	2	5.475	0.002	Yes

Lower Leg CSA

Source of Variation	DF	SS	MS	F	P
Group	2	3620992.528	1810496.264	0.862	0.445
Subject(Group)	13	27293552.687	2099504.053		
Time	1	1115701.974	1115701.974	49.817	<0.001
Group x Time	2	151932.515	75966.257	3.392	0.065
Residual	13	291148.821	22396.063		
Total	31	32362601.537	1043954.888		

Comparisons for factor: **Time**

Comparison	Diff of Means	p	q	P	P<0.050
PRE vs. POST	380.300	2	9.982	<0.001	Yes

Soleus Muscle

Source of Variation	DF	SS	MS	F	P
Group	2	303903.086	151951.543	0.453	0.645
Subject(Group)	13	4361606.838	335508.218		
Time	1	27941.041	27941.041	9.118	0.010
Group x Time	2	24242.317	12121.159	3.956	0.046
Residual	13	39836.417	3064.340		
Total	31	4749908.938	153222.869		

Comparisons for factor: **Time**

Comparison	Diff of Means	p	q	P	P<0.050
PRE vs. POST	60.183	2	4.270	0.010	Yes

Comparisons for factor: **Time within CON**

Comparison	Diff of Means	p	q	P	P<0.05
PRE vs. POST	138.456	2	5.002	0.004	Yes

Gastrocnemius Muscle

Source of Variation	DF	SS	MS	F	P
Group	2	348157.580	174078.790	0.448	0.648
Subject(Group)	13	5050937.749	388533.673		
Time	1	182499.081	182499.081	35.864	<0.001
Group x Time	2	6011.976	3005.988	0.591	0.568
Residual	13	66152.446	5088.650		
Total	31	5649200.034	182232.259		

Comparisons for factor: **Time**

Comparison	Diff of Means	p	q	P	P<0.050
PRE vs. POST	153.809	2	8.469	<0.001	Yes

Triceps Surae Muscle Group

Source of Variation	DF	SS	MS	F	P
Group	2	144984.313	72492.157	0.0438	0.957
Subject(Group)	13	21511907.820	1654762.140		
Time	1	378741.720	378741.720	41.803	<0.001
Group x Time	2	40600.750	20300.375	2.241	0.146
Residual	13	117783.251	9060.250		
Total	31	22161622.521	714891.049		

Comparisons for factor: **Time**

Comparison	Diff of Means	p	q	P	P<0.050
PRE vs. POST	221.577	2	9.144	<0.001	Yes

APPENDIX 3

KNEE EXTENSION NEUROMUSCULAR RAW DATA

& ANOVA TABLES

Knee Extension Neuromuscular Data (Page 1 of 2)

Subject	Group	Time	MVC (Nm)	%MUA	M-Wave (mV)	iEMG	PTT (avg.)	Rt (ms)	TPT (ms)	HRT (ms)
S5	CON	PRE	172	98.50	2.50	0.3548	27.30	51.33	78.92	65.42
S5	CON	POST	147	99.23	2.00	0.2580	26.05	53.75	83.08	62.08
S8	CON	PRE	229	94.01	0.64	0.9922	11.97	56.00	87.67	72.00
S8	CON	POST	146	96.15	0.17	4.8176	12.67	61.33	94.92	66.25
S9	CON	PRE	390	89.20	3.50	0.2011	56.84	63.08	96.67	58.42
S9	CON	POST	255	97.75	4.40	0.3523	37.33	61.25	91.33	56.58
S12	CON	PRE	408	96.72	4.00	0.2500	48.10	58.75	90.00	64.83
S12	CON	POST	309	99.55	3.90	0.3590	41.19	61.83	97.17	65.50
S18	CON	PRE	164	75.119	2.20	0.2636	24.30	49.50	78.83	73.42
S18	CON	POST	137	99.170	2.50	0.3512	23.73	54.08	79.33	55.83
	PRE	**MEAN**	**273**	**94.61**	**3.05**	**0.267**	**39.14**	**55.73**	**86.42**	**66.82**
		SEM	**53**	**2.03**	**0.42**	**0.032**	**7.93**	**2.46**	**3.41**	**2.71**
	POST	**MEAN**	**199**	**98.17**	**3.20**	**0.330**	**32.08**	**58.45**	**89.17**	**61.25**
		SEM	**35**	**0.78**	**0.57**	**0.024**	**4.25**	**1.85**	**3.43**	**2.18**
S4	EX	PRE	211	96.78	3.20	0.2500	21.06		73.50	73.83
S4	EX	POST	198	98.29	3.60	0.1767	27.45		88.17	63.67
S6	EX	PRE	217	78.25	2.00	0.1430	58.02	46.17	69.17	50.75
S6	EX	POST	206	83.15	1.10	0.3464	53.47	46.17	71.50	55.58
S11	EX	PRE	148	95.01	2.40	0.1308	29.20	47.75	72.83	67.75
S11	EX	POST	141	91.00	2.60	0.1550	26.80	48.83	74.58	71.50
S13	EX	PRE	344	95.74	3.80	0.2613	58.44	63.08	90.00	53.00
S13	EX	POST	289	91.86	3.70	0.3081	49.95	65.33	95.67	67.67
S15	EX	PRE	136	86.943	3.20	0.0894	26.73	46.33	72.08	61.50
S15	EX	POST	69	63.545	2.40	0.0479	27.54	49.17	77.08	70.33
S16	EX	PRE	195	95.53	2.30	0.1904	30.97	43.25	66.75	64.83
S16	EX	POST	170	91.02	3.10	0.1232	39.52	46.58	74.58	69.42
	PRE	**MEAN**	**223**	**92.26**	**2.82**	**0.177**	**37.40**	**49.32**	**74.06**	**61.94**
		SEM	**33**	**3.52**	**0.28**	**0.028**	**6.73**	**3.52**	**3.35**	**3.60**
	POST	**MEAN**	**201**	**91.07**	**2.75**	**0.193**	**37.46**	**51.22**	**80.26**	**66.36**
		SEM	**25**	**2.40**	**0.39**	**0.046**	**4.93**	**3.58**	**3.88**	**2.43**

Knee Extension Neuromuscular Raw Data (page 2 of 2)

Subject	Group	Time	MVC (Nm)	%MUA	M-Wave (mV)	PTT (Nm)	iEMG	PTT (avg.)	Rt (ms)	TPT (ms)	HRT (ms)
S1	WHY+EX	PRE	158	93.55	2.50	22.26	0.1380	22.00	55.08	82.58	57.75
S1	WHY+EX	POST	158	92.54	3.60	28.53	0.2608	27.75	52.33	85.17	66.17
S7	WHY+EX	PRE	251	85.18	3.60	39.57	0.1339	39.32	53.33	80.58	60.17
S7	WHY+EX	POST	235	84.42	4.20	49.96	0.2976	49.26	47.33	81.42	71.17
S10	WHY+EX	PRE	261	98.20	3.20	27.68	0.2497	27.57	48.88	73.63	73.38
S10	WHY+EX	POST	226	99.67	3.40	34.13	0.2391	33.73	46.17	70.50	71.75
S14	WHY+EX	PRE	135		2.40	31.78	0.1292	31.42	52.33	91.00	75.17
S14	WHY+EX	POST	128		2.40	29.47	0.1800	29.02	58.42	92.00	72.75
S17	WHY+EX	PRE	172	95.08	2.60	44.4	0.2327	44.39	50.42	80.33	64.00
S17	WHY+EX	POST	176	95.83	3.10	38.94	0.1852	38.74	49.33	78.83	78.67
S19	WHY+EX	PRE	147	94.03	2.90	13.68	0.2817	13.44	49.50	74.75	67.25
S19	WHY+EX	POST	99	89.98	2.50	19.3	0.1984	19.00	45.75	72.75	71.00
	PRE	**MEAN**	**187**	**93.21**	**2.87**	**33.14**	**0.194**	**32.94**	**51.59**	**80.48**	**66.28**
		SEM	**22**	**2.16**	**0.19**	**3.99**	**0.028**	**4.02**	**0.98**	**2.55**	**2.86**
	POST	**MEAN**	**170**	**92.49**	**3.20**	**36.21**	**0.227**	**35.70**	**49.89**	**80.11**	**71.92**
		SEM	**22**	**2.59**	**0.28**	**3.91**	**0.019**	**3.90**	**1.97**	**3.25**	**1.64**

Knee Extension %MUA

Source of Variation	DF	SS	MS	F	P
Group	2	41.390	20.695	0.316	0.735
Subject(Group)	11	719.784	65.435		
Time	1	33.253	33.253	1.640	0.227
Group x Time	2	111.923	55.962	2.759	0.107
Residual	11	223.090	20.281		
Total	27	1134.523	42.019		

Knee Extension Peak Twitch Torque (N·m)

Source of Variation	DF	SS	MS	F	P
Group	2	53.671	26.836	0.0953	0.910
Subject(Group)	12	3378.077	281.506		
Time	1	14.629	14.629	0.572	0.464
Group x Time	2	112.055	56.027	2.192	0.154
Residual	12	306.652	25.554		
Total	29	3857.107	133.004		

Vastus Medialis iEMG (mV)

Source of Variation	DF	SS	MS	F	P
Group	2	0.0556	0.0278	5.096	0.023
Subject(Group)	13	0.0710	0.00546		
Time	1	0.0131	0.0131	2.434	0.145
Group x Time	2	0.00136	0.000678	0.126	0.883
Residual	12	0.0648	0.00540		
Total	30	0.208	0.00693		

Knee Extension Twitch Rise Time (Rt)

Source of Variation	DF	SS	MS	F	P
Group	2	332.577	166.289	2.750	0.104
Subject(Group)	12	725.590	60.466		
Time	1	1.538	1.538	0.715	0.414
Group x Time	2	52.480	26.240	12.197	0.001
Residual	12	25.817	2.151		
Total	29	1138.002	39.241		

Comparisons for factor: **Time within CON**

Comparison	Diff of Means	p	q	P	P<0.05
POST vs. PRE	2.717	2	4.142	0.013	Yes

Comparisons for factor: **Time within EX**

Comparison	Diff of Means	p	q	P	P<0.05
POST vs. PRE	1.900	2	2.897	0.063	No

Comparisons for factor: **Time within WHY+EX**

Comparison	Diff of Means	p	q	P	P<0.05
PRE vs. POST	3.258	2	4.967	0.004	Yes

Knee Extension Twitch Half Relaxation Time (HRt)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Group	2	227.390	113.695	2.132	0.158
Subject(Group)	13	693.285	53.330		
Time	1	48.300	48.300	2.396	0.146
Group x Time	2	250.292	125.146	6.209	0.013
Residual	13	262.038	20.157		
Total	31	1489.784	48.058		

Comparisons for factor: **Time within CON**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
PRE vs. POST	5.567	2	2.772	0.072	No

Comparisons for factor: **Time within EX**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
POST vs. PRE	7.333	2	3.652	0.023	Yes

Comparisons for factor: **Time within WHY+EX**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
POST vs. PRE	5.632	2	3.073	0.049	Yes

APPENDIX 4

PLANTAR FLEXION NEUROMUSCULAR RAW DATA

& ANOVA TABLES

Plantar Flexion Neuromuscular Data (Page 1 of 2)

Subject	Group	Time	MVC (Nm)	%MUA	M-Wave (mV)	iEMG	PTT (avg.)	Rt (ms)	TPT (ms)	HRT (ms)
S5	CON	PRE	113	93.73	3.90	0.1849	21.07	56.92	95.33	91.17
S5	CON	POST	121	96.39	4.50	0.1916	19.48	69.67	111.17	110.75
S8	CON	PRE	188	96.59	2.60	0.2015	25.38	70.00	115.75	107.67
S8	CON	POST	138	98.04	2.90	0.2114	22.95	93.42	153.42	163.42
S9	CON	PRE	215	97.68	6.40	0.1953		67.67	109.42	100.67
S9	CON	POST	164	91.42	7.10	0.1318		78.83	121.00	113.75
S12	CON	PRE	254		3.10	0.1835	29.68	68.75	109.08	104.75
S12	CON	POST	196		3.70	0.1649	25.19	76.00	118.17	116.33
S18	CON	PRE	165	93.61	3.40	0.2935	16.34	80.42	140.00	99.33
S18	CON	POST	116	96.87	5.00	0.2640	18.93	87.08	143.50	128.08
	PRE	**MEAN**	**206**	**95.40**	**3.88**	**0.212**	**23.12**	**68.75**	**113.92**	**100.72**
		SEM	**19**	**1.03**	**0.66**	**0.021**	**2.86**	**3.73**	**7.32**	**2.81**
	POST	**MEAN**	**154**	**95.68**	**4.64**	**0.193**	**21.64**	**81.00**	**129.45**	**126.47**
		SEM	**17**	**1.46**	**0.71**	**0.022**	**1.48**	**4.18**	**8.08**	**9.69**
S4	EX	PRE	164	99.31	5.20	0.2327	21.40	62.92	103.58	100.25
S4	EX	POST	143	93.05	4.90	0.1804	21.28	73.08	116.25	120.33
S6	EX	PRE	155	88.34	2.90	0.0831	29.88	48.25	74.67	106.25
S6	EX	POST	172	86.78	3.20	0.1225	29.24	51.83	81.92	103.50
S11	EX	PRE	153	91.90	2.30	0.2922	23.35	63.25	107.17	119.00
S11	EX	POST	144	95.07	3.00	0.2010	22.83	65.75	114.00	124.75
S13	EX	PRE	216	92.87	1.30	0.1954	27.86	72.50	115.58	114.83
S13	EX	POST	214	99.31	0.62	0.2661	28.00	75.08	118.67	127.92
S15	EX	PRE	85	57.335	4.00	0.0733	24.49	67.42	114.08	107.00
S15	EX	POST	72	68.542	1.70	0.1429	18.98	76.92	123.50	143.67
S16	EX	PRE	157	99.45	3.50	0.1571	22.35	58.58	96.08	91.92
S16	EX	POST	144	95.44	2.00	0.5900	23.69	67.75	117.25	108.25
	PRE	**MEAN**	**155**	**94.38**	**3.58**	**0.175**	**24.97**	**62.15**	**101.86**	**106.54**
		SEM	**17**	**7.04**	**0.50**	**0.043**	**1.65**	**3.38**	**6.17**	**3.99**
	POST	**MEAN**	**148**	**93.93**	**3.28**	**0.152**	**25.01**	**68.40**	**111.93**	**121.40**

Plantar Flexion Neuromuscular Data (Page 2 of 2)

Subject	Group	Time	MVC (Nm)	%MUA	M-Wave (mV)	iEMG	PTT (avg.)	Rt (ms)	TPT (ms)	HRT (ms)
S1	WHY+EX	PRE	123	88.58	4.90	0.1245	21.02	66.75	106.33	100.58
S1	WHY+EX	POST	145	99.04	5.00	0.1336	24.59	75.08	119.08	103.92
S7	WHY+EX	PRE	209	91.12	3.20	0.2206	24.16	57.33	89.67	121.42
S7	WHY+EX	POST	205	94.49	5.90	0.2085	27.85	60.67	98.17	98.58
S10	WHY+EX	PRE	173	99.57	3.60	0.2308	18.94	87.08	135.83	126.08
S10	WHY+EX	POST	126	94.77	3.30	0.2312	16.47	74.42	118.50	119.08
S14	WHY+EX	PRE	130	98.05	2.60	0.0827	26.16	89.00	143.00	111.25
S14	WHY+EX	POST	122	96.16	3.00	0.1057	24.13	95.25	153.17	136.50
S17	WHY+EX	PRE	89	94.45	2.50	0.2496	15.17	77.92	127.50	114.33
S17	WHY+EX	POST	137	99.79	3.90	0.2126	16.54	71.08	119.75	105.83
S19	WHY+EX	PRE	127	94.10	4.20	0.2117	17.48	74.42	124.00	124.83
S19	WHY+EX	POST	103	96.59	4.10	0.2683	18.41	80.00	132.58	138.17
	PRE	**MEAN**	**142**	**94.31**	**3.50**	**0.187**	**20.49**	**75.42**	**121.06**	**116.42**
		SEM	**17**	**1.68**	**0.40**	**0.027**	**1.69**	**4.93**	**8.07**	**3.96**
	POST	**MEAN**	**140**	**96.80**	**4.26**	**0.193**	**21.33**	**76.08**	**123.54**	**117.01**
		SEM	**18**	**0.89**	**0.54**	**0.025**	**1.97**	**4.65**	**7.45**	**6.99**

Plantar Flexion %MUA

Source of Variation	DF	SS	MS	F	P
Group	2	13.068	6.534	0.460	0.642
Subject(Group)	12	170.287	14.191		
Time	1	4.357	4.357	0.335	0.573
Group x Time	2	12.932	6.466	0.498	0.620
Residual	12	155.845	12.987		
Total	29	358.487	12.362		

Plantar Flexion Peak Twitch Torque (N·m)

Source of Variation	DF	SS	MS	F	P
Group	2	91.569	45.784	1.386	0.287
Subject(Group)	12	396.297	33.025		
Time	1	0.289	0.289	0.108	0.748
Group x Time	2	6.493	3.246	1.215	0.331
Residual	12	32.058	2.672		
Total	29	526.432	18.153		

Plantar Flexion iEMG (mV)

Source of Variation	DF	SS	MS	F	P
Group	2	0.00277	0.00138	0.124	0.884
Subject(Group)	14	0.156	0.0112		
Time	1	0.00406	0.00406	0.626	0.442
Group x Time	2	0.0143	0.00717	1.105	0.358
Residual	14	0.0909	0.00649		
Total	33	0.269	0.00816		

Plantar Flexion Twitch Rise Time (Rt)

Source of Variation	DF	SS	MS	F	P
Group	2	792.258	396.129	2.247	0.142
Subject(Group)	14	2467.664	176.262		
Time	1	344.401	344.401	15.898	0.001
Group x Time	2	183.161	91.580	4.227	0.037
Residual	14	303.292	21.664		
Total	33	4056.892	122.936		

Comparisons for factor: **Time**

Comparison	Diff of Means	p	q	P	P<0.050
POST vs. PRE	6.389	2	5.639	0.001	Yes

Comparisons for factor: **Time within CON**

Comparison	Diff of Means	p	q	P	P<0.05
POST vs. PRE	12.250	2	5.885	0.001	Yes

Comparisons for factor: **Time within EX**

Comparison	Diff of Means	p	q	P	P<0.05
POST vs. PRE	6.250	2	3.289	0.036	Yes

Comparisons for factor: **Time within WHY+EX**

Comparison	Diff of Means	p	q	P	P<0.05
POST vs. PRE	0.667	2	0.351	0.808	No

Plantar Flexion Twitch Half Relaxation Time (HRt)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Group	2	66.815	33.408	0.128	0.881
Subject(Group)	14	3659.219	261.373		
Time	1	1591.994	1591.994	12.125	0.004
Group x Time	2	878.277	439.138	3.345	0.065
Residual	14	1838.167	131.298		
Total	33	7885.485	238.954		

APPENDIX 5

KNEE EXTENSION MVC and SPECIFIC STRENGTH

& ANOVA TABLES

Knee Extension MVC and Specific Strength: SS = MVC/(CSAxheight) ($N{\cdot}m/cm^2{*}m$)

Subject	Group	Height (m)	MVC (N·m)- Pre	CSA (cm^2) Pre	SS PRE	MVC (N·m) Post	CSA (cm^2) Post	MVC % Change	SS POST	SS – % Change
S5	CON	1.630	172.00	43.039	2.452	147.00	43.151	-14.535	2.090	-14.757
S8	CON	1.800	229.00	49.281	2.582	146.00	49.069	-36.245	1.653	-35.968
S9	CON	1.840	390.00	106.738	1.986	255.00	91.916	-34.615	1.508	-24.072
S12	CON	1.920	408.00	88.762	2.394	309.00	80.333	-24.265	2.003	-16.318
S18	CON	1.590	164.00	48.164	2.142	137.00	41.090	-16.463	2.097	-2.083
	MEAN	**1.756**	**272.60**	**67.197**	**2.311**	**198.80**	**61.112**	**-25.225**	**1.870**	**-18.640**
	SEM	**0.063**	**52.88**	**12.836**	**0.108**	**35.07**	**10.457**	**4.481**	**0.122**	**5.587**
S4	EX	1.700	211.00	56.222	2.208	198.00	57.783	-6.161	2.016	-8.697
S6	EX	1.770	217.00	95.453	1.284	206.00	99.068	-5.069	1.175	-8.533
S11	EX	1.610	148.00	55.449	1.658	141.00	56.263	-4.730	1.557	-6.108
S13	EX	1.980	344.00	87.449	1.987	289.00	91.559	-15.988	1.594	-19.760
S15	EX	1.660	135.84	60.98	1.342	68.52	63.97	-49.558	0.645	-51.919
S16	EX	1.620	195.00	63.187	1.905	170.00	66.364	-12.821	1.581	-16.994
	MEAN	**1.736**	**223.000**	**69.789**	**1.808**	**200.800**	**72.501**	**-8.954**	**1.584**	**-12.018**
	SEM	**0.068**	**32.581**	**8.331**	**0.158**	**24.838**	**8.867**	**2.293**	**0.133**	**2.672**
S1	WHY+EX	1.680	158.00	50.745	1.853	158.00	47.450	0.000	1.982	6.944
S7	WHY+EX	1.830	251.00	86.150	1.592	235.00	85.053	-6.375	1.510	-5.167
S10	WHY+EX	1.720	261.00	61.341	2.474	226.00	62.040	-13.410	2.118	-14.386
S14	WHY+EX	1.740	135.00	55.658	1.394	128.00	58.871	-5.185	1.250	-10.359
S17	WHY+EX	1.640	172.00	53.439	1.963	176.00	59.435	2.326	1.806	-7.997
S19	WHY+EX	1.630	147.00	49.060	1.838	99.00	49.410	-32.653	1.229	-33.130
	MEAN	**1.707**	**187.33**	**59.399**	**1.852**	**170.33**	**60.377**	**-9.216**	**1.649**	**-10.683**
	SEM	**0.030**	**22.32**	**5.629**	**0.150**	**21.87**	**5.484**	**5.194**	**0.154**	**5.373**

Knee Extension Isometric MVC (N·m)

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Group	2	17997.208	8998.604	0.854	0.448
Subject(Group)	13	137040.667	10541.590		
Time	1	11266.765	11266.765	23.107	<0.001
Group x Time	2	5130.075	2565.037	5.261	**0.021**
Residual	13	6338.800	487.600		
Total	31	177091.875	5712.641		

Comparisons for factor: **Time**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
PRE vs. POST	37.667	2	6.798	<0.001	Yes

Comparisons for factor: **Time within CON**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0.05**
PRE vs. POST	73.800	2	7.473	<0.001	Yes

Knee Extension Specific Strength

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Group	2	0.930	0.465	2.546	0.117
subjects(Group)	13	2.374	0.183		
Time	1	0.665	0.665	21.847	<0.001
Group x Time	2	0.0900	0.0450	1.479	0.264
Residual	13	0.395	0.0304		
Total	31	4.434	0.143		

APPENDIX 6

PLANTAR FLEXION MVC and SPECIFIC STRENGTH

& ANOVA TABLES

Plantar Flexion MVC (N·m) and Specific Strength: SS = MVC/(CSA x height) (N·m/cm^2*m)

Subject	Group	Height (m)	MVC (N*m) Pre	CSA (cm2) Pre	SS PRE	MVC (N*m) Post	CSA (cm2) Post	MVC % Change	SS POST	SS % Change
S5	CON	1.63	113			121				
S8	CON	1.800	188	37.267	2.803	138	36.138	-26.596	2.121	-24.303
S9	CON	1.840	215.000	41.433	2.820	164	35.699	-23.721	2.497	-11.468
S12	CON	1.920	254	44.630	2.964	196	41.100	-22.835	2.484	-16.207
S18	CON	1.590	165	36.152	2.870	116	33.364	-29.697	2.187	-23.822
	MEAN	**1.788**	**205.500**	**39.870**	**2.864**	**153.500**	**36.575**	**-25.712**	**2.322**	**-18.950**
	SEM	**0.070**	**19.125**	**1.952**	**0.036**	**17.231**	**1.627**	**1.552**	**0.098**	**3.108**
S4	EX	1.700	164	34.502	2.796	143	33.812	-12.805	2.488	-11.025
S6	EX	1.770	155	57.533	1.522	172	52.895	10.968	1.837	20.697
S11	EX	1.610	153	30.829	3.083	144	29.898	-5.882	2.992	-2.952
S13	EX	1.980	216	50.378	2.165	214	49.518	-0.926	2.183	0.795
S15	EX	1.660	85	21.194	2.416	72	19.199	-15.294	2.259	-6.492
S16	EX	1.620	157	40.640	2.385	144	38.852	-8.280	2.288	-4.060
	MEAN	**1.723**	**155.000**	**39.179**	**2.394**	**148.167**	**37.362**	**-5.370**	**2.341**	**-0.506**
	SEM	**0.057**	**17.039**	**5.414**	**0.220**	**18.943**	**5.130**	**3.869**	**0.156**	**4.533**
S1	WHY+EX	1.680	123	35.784	2.046	145	33.903	17.886	2.546	24.425
S7	WHY+EX	1.830	209	47.807	2.389	205	47.257	-1.914	2.370	-0.772
S10	WHY+EX	1.720	173	34.651	2.903	126	33.485	-27.168	2.188	-24.631
S14	WHY+EX	1.740	130	42.481	1.759	122	40.776	-6.154	1.720	-2.230
S17	WHY+EX	1.640	89	33.67	1.612	137	31.86	53.933	2.622	62.695
S19	WHY+EX	1.630	127	31.38	2.483	103	29.29	-18.898	2.157	-13.112
	MEAN	**1.720**	**152.400**	**37.629**	**2.316**	**140.200**	**36.095**	**-7.249**	**2.196**	**-3.264**
	SEM	**0.033**	**16.792**	**2.544**	**0.195**	**17.520**	**2.723**	**7.726**	**0.138**	**8.147**

Plantar Flexion Isometric MVC (N·m)

Source of Variation	DF	SS	MS	F	P
Group	2	5544.850	2772.425	0.889	0.437
Subject(Group)	12	37441.017	3120.085		
Time	1	4091.136	4091.136	27.834	<0.001
Group x Time	2	2716.850	1358.425	9.242	0.004
Residual	12	1763.817	146.985		
Total	29	50669.867	1747.237		

Comparisons for factor: **Time**

Comparison	Diff of Means	p	q	P	P<0.050
PRE vs. POST	23.678	2	7.461	<0.001	Yes

Comparisons for factor: **Time within CON**

Comparison	Diff of Means	p	q	P	P<0.05
PRE vs. POST	52.000	2	8.578	<0.001	Yes

Plantar Flexion Specific Strength

Source of Variation	DF	SS	MS	F	P
Group	2	0.616	0.308	1.273	0.315
Subject(Group)	12	2.904	0.242		
Time	1	0.416	0.416	8.996	0.011
Group x Time	2	0.314	0.157	3.396	0.068
Residual	12	0.555	0.0462		
Total	29	4.708	0.162		

APPENDIX 7

NUTRITIONAL INFORMATION AND AMINO ACID PROFILE FOR

PROTIENT 9500: INSTANT WHEY PROTEIN ISOLATE

PROTIENT 9500: Instant Whey Protein Isolate

Typical Nutritional Information

Values are per 100 grams of product

Calories	365 kcal	Lactose	2.00 g
Calories from Fat	2.25 kcal	Protein	91.0 g (d.b.)
Total Fat	2 g	Phosphorous	214 mg
Saturated Fat	1 g	Sodium	150 mg
Monounsaturated Fat	0.8 g	Calcium	433 mg
Polyunsaturated Fat	0.2 g	Magnesium	64.1 mg
Trans Fatty Acids	0.02 g	Vitamin A	<50 IU
Cholesterol	8 mg	Potassium	484 mg
Ash	2.80 g	Iron	0.48 mg
Moisture	5.0 g	Riboflavin	0.13 mg
Total Carbohydrate	4.73 g		

Typical Amino Acid Profile

Values are milligrams amino acid per 100 grams product

Alanine	4500	Lysine	8530
Arginine	1730	Methionine	1820
Aspartic Acid	9890	Phenylalanine	2830
Cystine	2060	Proline	5850
Glutamic Acid	15800	Serine	4070
Glycine	1480	Threonine	6420
Histidine	1540	Tryptophan	1490
Isoleucine	6170	Tyrosine	2760
Leucine	9440	Valine	5470

Updated 10/05 *from vE0105a*

Contains: Whey Protein Isolate, Soy Lecithin.

APPENDIX 8

DIETARY RECORDS & PROTEIN INTAKE RAW DATA

AND ANOVA TABLES

Dietary Records Raw Data

Subject	Time	Group	Total Calories	%CHO	%PRO	%FAT	% Alcohol
S1	PRE	WHY+EX	2217	61	15	28	0
S4	PRE	EX	2333	55	14	34	0
S5	PRE	CON	1286	46	22	33	0
S6	PRE	EX	2697	50	14	38	0
S7	PRE	WHY+EX	1862	65	14	24	0
S8	PRE	CON	1324	69	13	24	0
S9	PRE	CON	3032	48	16	36	2
S10	PRE	WHY+EX	1130	51	20	24	7
S11	PRE	EX					
S12	PRE	CON	1353	52	17	32	0
S13	PRE	EX					
S14	PRE	WHY+EX	1760	54	16	32	1
S15	PRE	EX	1318	59	14	28	0
S16	PRE	EX	1948	55	18	28	0
S17	PRE	WHY+EX	1605	53	10	30	8
S18	PRE	CON	2576	62	11	25	5
S19	PRE	WHY+EX	2346	46	18	35	3
S1	POST	WHY+EX	2225	59	21	22	0
S4	POST	EX	1857	51	19	31	0
S5	POST	CON	1640	66	18	17	0
S6	POST	EX	2249	56	14	31	1
S7	POST	WHY+EX	1682	51	22	25	4
S8	POST	CON	1729	67	12	25	0
S9	POST	CON	2311	56	16	30	0
S10	POST	WHY+EX	2054	47	16	37	0
S11	POST	EX					
S12	POST	CON	1598	49	20	32	0
S13	POST	EX					
S14	POST	WHY+EX	1395	55	15	27	6
S15	POST	EX	1195	58	15	28	0
S16	POST	EX	2486	62	16	25	0
S17	POST	WHY+EX	3040	54	10	23	15
S18	POST	CON	2000	62	12	28	1
S19	POST	WHY+EX	2724	58	15	26	4

Protein Intake for WHY+EX Group

Subject	Group	Time	Weight (kg)	Grams of Protein/day	Avg. Protein Intake (g/kg/d)	% Change in Protein Intake
S1	WHY+EX	DR1	59	80	1.36	47.9
S1	WHY+EX	DR2		119	2.02	
S7	WHY+EX	DR1	77	65	0.85	43.0
S7	WHY+EX	DR2		93	1.21	
S10	WHY+EX	DR1	93.8	57	0.60	47.1
S10	WHY+EX	DR2		83	0.89	
S14	WHY+EX	DR1	76	69	0.91	-23.8
S14	WHY+EX	DR2		53	0.69	
S17	WHY+EX	DR1	74	86	1.17	14.8
S17	WHY+EX	DR2		99	1.34	
S19	WHY+EX	DR1	75.8	104	1.37	-2.6
S19	WHY+EX	DR2		102	1.34	
	PRE	**MEAN**	75.9	77	1.04	21.1
		SEM	4.5	7	0.13	12.2
	POST	**MEAN**		91	1.25	
		SEM		9	0.19	

Average Kilocalories/day

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Group	2	98035.901	49017.951	0.101	0.905
Subject(Group)	12	5845274.456	487106.205		
Time	1	26541.991	26541.991	0.149	0.706
Group x Time	2	379612.056	189806.028	1.068	0.374
Residual	12	2133133.009	177761.084		
Total	29	8521287.062	293837.485		

Protein Intake for WHY+EX Group

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Subjects	5	1.288	0.258		
Between Treatments	1	0.124	0.124	2.646	0.165
Residual	5	0.234	0.0469		
Total	11	1.646			