

**RESISTANCE TRAINING-INDUCED CHANGES IN HUMAN MUSCLE
PROTEIN SYNTHESIS AND FIBRE MORPHOLOGY**

**RESISTANCE TRAINING-INDUCED CHANGES IN HUMAN MUSCLE
PROTEIN SYNTHESIS AND FIBRE MORPHOLOGY**

By

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ABSTRACT

Muscle proteins are in a continuous state of recycling. This process involves a balance between synthesis and breakdown. These opposing processes dictate muscle protein gains and losses. Muscle hypertrophy occurs when synthesis exceeds breakdown. In order for the accretion of new muscle proteins, a chronic state of net positive muscle protein balance (synthesis > breakdown) is required.

Resistance exercise is a potent stimulus of protein turnover and the combined effects of exercise and feeding have shown to be necessary for net protein anabolism. Resistance training has been reported to increase muscle strength and induce changes in skeletal muscle morphology. These positive strength adaptations include muscle fibre hypertrophy and a shift in fibre type from IIX to IIA.

Previous investigations of resistance training-induced changes in muscle protein synthesis and fibre morphology have utilized cross-sectional or longitudinal, bilateral training designs. Thus, the purpose of this study was to investigate the effects of a progressive eight week unilateral leg resistance training program on skeletal muscle morphology, and resting and exercise-stimulated mixed muscle protein fractional synthesis rate (FSR). Eight young men performed two training sessions each week, and each session consisted of four sets of knee extension (KE) and four sets of leg press (LP) at 80% 1 repetition maximum (1 RM). Needle biopsies from the vastus lateralis muscle of the trained (T) leg were taken before and after training and analyzed for fibre composition, cross-sectional area (CSA), and myosin heavy chain (MHC) content. Muscle protein FSR was determined using a primed constant stable isotope infusion of [¹³C₆]-phenylalanine in both

the T and untrained (UT) legs. Training induced type IIX and IIA fibre hypertrophy ($P < 0.05$) with no change for type I fibre CSA. There was no significant change in histochemically determined fibre composition or MHC content. After training, 1RM strength of the T leg significantly increased compared to baseline values ($P < 0.01$). At rest, FSR was significantly elevated in the T versus the UT leg ($P < 0.01$). Following an acute bout of resistance exercise, which was performed at the same relative intensity (80% 1 RM) for the T and UT legs, FSR was greater in the UT versus the T leg ($P < 0.01$). There was a lower exercise-induced increase in muscle FSR in the T versus the UT leg compared to their respective resting values (T: $P = 0.08$, UT: $P < 0.01$). These data show that resistance training resulted in significant muscle fibre hypertrophy and elevated rate of muscle protein synthesis at rest. In addition, the acute response to resistance exercise was characterized by an attenuated rise in muscle protein FSR in the T versus the UT leg. We conclude that resistance training markedly attenuates the acute muscle protein synthetic response following resistance exercise, even when loads are matched at the same relative intensity.

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Chapter I

LITERATURE REVIEW

1.1. Introduction

Body proteins are in a continuous state of remodeling. This process, termed protein turnover, is a balance between protein synthesis and breakdown (Figure 1). A stimulator of muscle protein turnover is resistance exercise or weightlifting, which increases both synthesis and breakdown after a single bout (Biolo *et al.*, 1995b; Phillips *et al.*, 1997). In order for muscle growth (hypertrophy) to occur, a net positive muscle protein balance (synthesis minus breakdown) is required (i.e. synthetic rates must exceed degradation).

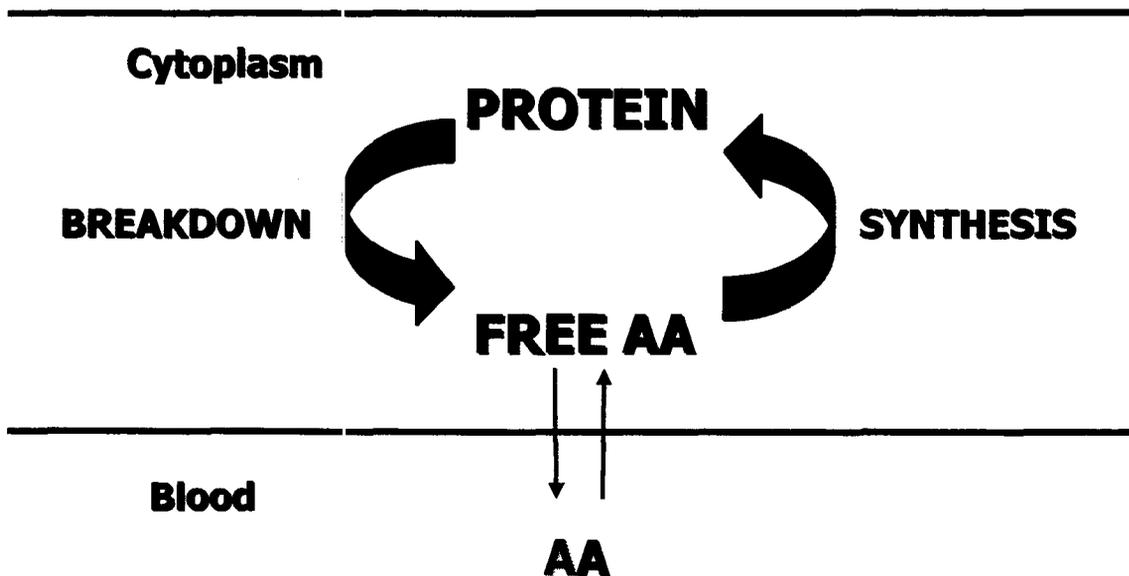


Figure 1. Schematic representation of protein turnover. AA: amino acids

Skeletal muscle is one of the best examples of a tissue that has the inherent ability to adapt to external stimuli. In studies that have examined the combined effects of resistance exercise and bed rest, which are associated with two opposing adaptations (hypertrophy in

the case of the former and atrophy with the latter), the prevention of muscle atrophy, maintenance of muscle strength (Bamman *et al.*, 1998) and muscle protein synthesis (Ferrando *et al.*, 1997) have been observed with minimal bouts of resistance exercise. Resistance training has been shown to induce numerous skeletal muscular adaptations, which include muscle fibre hypertrophy (increase in fibre cross-sectional area); increases in muscular strength and changes in protein metabolic characteristics. The additive effects of resistance exercise and feeding have been reported to result in a net positive protein balance (Tipton *et al.*, 1999a), which leads to muscle protein accretion and hypertrophy (Tipton *et al.*, 2001).

The rapid increases in protein synthesis following a single bout of resistance exercise have been hypothesized to be under post-transcriptional regulation (Chesley *et al.*, 1992; Phillips *et al.*, 1997). In other words, there seems to be greater translational efficiency for a given amount of mRNA transcript (Welle *et al.*, 1999). However, an attenuated exercise-induced stimulation of the muscle protein synthetic response has been reported to occur as a result of chronic resistance training (Phillips *et al.*, 1999; Farrell *et al.*, 1999a). This reduced response as a result of training is in accordance with the general principle of successful adaptation to a stressor; hence, as one becomes accustomed to a stressor, the physiological response due to that stressor is reduced.

There are three major fibre types (type I, IIA and IIX) in human skeletal muscle. These fibres are differentiated based on their respective MHC isoform (MHC I, MHC IIA and MHC IIX). As skeletal muscle undergoes repeated bouts of exercise over an extended period of time, alterations in the metabolic characteristics of the muscle fibres have been reported. A transition of type IIX fibres to IIA as a result of resistance training has been reported in

humans (Staron *et al.*, 1989). This transformation requires an up-regulation of MHC IIa mRNA expression following a bout of resistance exercise (Willoughby & Nelson, 2002), which may occur with long-term resistance training. Also, a shift towards a more oxidative fast type IIA fibre may be considered an advantageous strength adaptation of skeletal muscle due the greater fatigue resistance of these fibres.

Although the physiological adaptations of muscle hypertrophy and elevated muscle protein synthesis have been documented, the underlying mechanisms of these adaptations are poorly understood. An exercise-induced increase in muscle protein synthesis has been thought to be regulated to some degree at the initiation phase of mRNA translation. It has been suggested that eukaryotic initiation factor 2 (eIF2; Farrell *et al.*, 1999b) and 70-kDa S6 protein kinase (p70^{S6k}; Baar & Esser, 1999) are involved in the increased protein synthesis after resistance exercise. The maintenance of an enlarged myofibril (contractile unit of the muscle fibre) has been associated with an increased number of myonuclei derived from satellite cells (Kadi *et al.*, 1999; Kadi & Thornell, 2000). Hormonal responses have also been shown to play a significant role to muscle growth. For example, insulin seems to play a permissive role in muscle protein synthesis (Jacob *et al.*, 1996; Fedele *et al.*, 2000). In addition, locally expressed tissue growth factors, insulin-like growth factor-I (IGF-I) and mechanogrowth factor (MGF), both appear to be stretch-sensitive and may contribute to muscle fibre hypertrophy (Goldspink, 1999). Further investigations are necessary to elucidate the mechanisms underlying resistance-induced increases in muscle protein synthesis and muscle hypertrophy in humans.

1.2. Muscle Protein Turnover and Resistance Exercise

Mixed muscle protein synthesis (MPS) has shown to be elevated between 3-48 h post-exercise in fasted humans (Chesley *et al.*, 1992; Yarasheski *et al.*, 1993; Biolo *et al.*, 1995b; Biolo *et al.*, 1997; Phillips *et al.*, 1997; Phillips *et al.*, 1999; Yarasheski *et al.*, 1999). Also in the post-absorptive state, muscle protein breakdown (MPB) rates also increased disproportionately, which resulted in a net negative protein balance (synthesis minus breakdown) and an increased overall protein turnover (Biolo *et al.*, 1995b; Phillips *et al.*, 1997; Phillips *et al.*, 1999). Exercise alone improves protein balance, compared to resting values, but does not shift it to a net positive value (Biolo *et al.*, 1995b; Phillips *et al.*, 1997; Phillips *et al.*, 1999). Therefore, it has been suggested that physical exercise can reduce net muscle protein catabolism but does not promote net protein deposition, at least not in the postabsorptive state (Biolo *et al.*, 1995b). In the fasted state, free intracellular amino acids for protein synthesis are only available via protein degradation. Thus, a decrease in intracellular amino acids below post-absorptive levels, due to increased protein synthesis, has been hypothesized to stimulate protein breakdown to restore amino acids to normal levels (Biolo *et al.*, 1995b). Increases in protein degradation after exercise reported by Biolo *et al.* (1995b) were not seen with amino acid administration (Biolo *et al.*, 1997). It was suggested that the amino acid infusion attenuated the increase in protein breakdown that normally takes place after exercise (Biolo *et al.*, 1997). A recent study reported that during rest and after the consumption of an amino acid and carbohydrate mixture, protein breakdown was seen to be lower compared to postabsorptive state (Volpi *et al.*, 2000). Thus, an inhibitory effect of systemic hyperaminoacidemia on protein breakdown is thought to be the mechanism that can

explain the lack of an increase in MPB after exercise and results in a net positive protein balance (Rasmussen *et al.*, 2000).

In order for muscle mass to increase, a net positive protein balance is chronically required (i.e. net synthesis exceeds breakdown for the entire training period). Resistance exercise has been shown to elevate mixed muscle protein synthesis (Chesley *et al.*, 1992; Biolo *et al.*, 1995b; Phillips *et al.*, 1997; Phillips *et al.*, 1999), myofibrillar protein synthesis (Welle *et al.*, 1999) and myosin heavy chain synthesis (Hasten *et al.*, 2000). The accretion of myofibrillar proteins results in an increase in fibre diameter. However, an additional determinant of muscle protein synthesis is the availability of nutrients (i.e. amino acids as substrates of synthesis). Administration of exogenous amino acids is one method of increasing amino acid availability and increasing muscle protein synthesis (Volpi *et al.*, 1998). Net positive protein balance has been achieved post-exercise through intravenous (Biolo *et al.*, 1997) or oral (Tipton *et al.*, 1999a; Tipton *et al.*, 1999b; Rasmussen *et al.*, 2000; Tipton *et al.*, 2001) administration of amino acids. Exogenous administration of amino acids increases inward intracellular amino acid transport at rest and after exercise, thereby increasing intracellular amino acid concentrations and availability for protein synthesis (Biolo *et al.*, 1997; Volpi *et al.*, 1998; Tipton *et al.*, 1999a; Rasmussen *et al.*, 2000). The effect of exercise with post-exercise amino acid provision resulted in an additive or synergistic effect, enhancing muscle protein synthesis beyond the state of ingesting amino acids alone at rest (Biolo *et al.*, 1997; Tipton *et al.*, 1999a; Rasmussen *et al.*, 2000; Tipton *et al.*, 2001).

It seems that non-essential amino acids are not necessary to stimulate net muscle protein synthesis and essential amino acids are sufficient for net protein synthesis (Smith *et*

al., 1998; Tipton *et al.*, 1999a; Tipton *et al.*, 1999b; Rasmussen *et al.*, 2000; Tipton *et al.*, 2001; Borsheim *et al.*, 2002). It has recently been reported that additional carbohydrate provided no advantage to essential amino acids alone in stimulating muscle protein synthesis after resistance exercise (Borsheim *et al.*, 2002). Therefore, the provision of essential amino acids appears to be the primary determinant for muscle protein anabolism after exercise.

The timing of amino acid supplementation can alter the anabolic response to resistance exercise. The response of net muscle protein synthesis to an essential amino acid solution immediately before resistance exercise has been reported to be greater than post-exercise supplementation (Tipton *et al.*, 2001). This was primarily due to an increase in the delivery of amino acids to the exercised muscle as a result of increased leg blood flow (Tipton *et al.*, 2001). Increased blood flow to working muscles may be a contributing factor to elevated protein synthesis. Previous reports have shown that an increased amino acid delivery (i.e. blood flow \times [amino acids]) is associated with increased transport of amino acids into muscle, thus increasing the intracellular amino acid availability and possibly stimulating protein synthesis (Biolo *et al.*, 1995b; Biolo *et al.*, 1997; Volpi *et al.*, 1998).

1.3. Muscle Protein Synthesis and Resistance Training

The rate of mixed muscle protein synthesis is increased after an acute bout of exercise in both humans and animals (Biolo *et al.*, 1995b; Phillips *et al.*, 1997; Farrell *et al.*, 1998; Phillips *et al.*, 1999; Biolo *et al.*, 1999; Farrell *et al.*, 1999b). However, it has been suggested that lack of a significant response in muscle protein synthesis to resistance exercise in humans reported in some studies may be due to the initial training status of the subjects (Tipton *et al.*, 1996; Roy *et al.*, 1997). Subjects who have not shown a marked increase in

muscle protein synthesis (Roy *et al.*, 1997) were already resistance exercise-trained and, hence, what might have been observed is a biological adaptation to the stress of exercise (Rennie & Tipton, 2000). Elevations in rates of mixed muscle protein synthesis are considered to be an adaptation provided the resistance exercise (stimulus) is sufficient to stress the tissue beyond its existing capacity. Chesley *et al.* (1992) reported a 50% increase in muscle protein synthesis 4 h post-exercise, in trained men. These previously trained subjects performed a total of 12 sets of 6-12 repetitions at 80% 1 repetition maximum (1 RM) of elbow flexor exercises. However, in studies which did not show a significant effect on muscle protein synthesis exercised 6 sets at 65% 1 RM during a whole body routine (Tipton *et al.*, 1996), or 8 sets at 85% 1 RM (Roy *et al.*, 1997) of the sampled muscle group. These findings indicate that, in trained individuals, a heavy bout of resistance exercise (high intensity and volume) might be required to stimulate muscle protein synthesis.

Resistance exercise has been reported to result in hypertrophy of the trained muscles (Ploutz *et al.*, 1994; McCall *et al.*, 1996; Hakkinen *et al.*, 2001). Muscle hypertrophy is a result of the accretion of newly synthesized myofibrillar proteins in the resting recovering muscle. An interesting but under-investigated issue is how resistance training affects muscle protein turnover at rest and post-exercise. Rennie and Tipton (2000) hypothesized that if muscle protein synthesis responds to a repeated stimulus of a constant magnitude (absolute workload) in the same manner as other physiological systems (i.e. maximum oxygen uptake capacity, VO_{2max}), then it is likely that a series of training stimuli would result in progressively reduced responses. Therefore, after training, an increased stimulus would be required in order to initiate a response similar to that observed before training. The results from a cross-sectional study of resistance-trained and untrained humans support this idea

(Phillips *et al.*, 1999). A unilateral leg exercise model was used to measure mixed muscle protein synthesis at rest (i.e. control or non-exercised leg) and after an acute bout of exercise in the contralateral leg at same relative workload for both trained and untrained subjects. The response of muscle protein synthesis was reduced in the exercised leg of the trained subjects compared to the untrained subjects (Phillips *et al.*, 1999). However, there were no significant differences in resting muscle protein synthesis between groups, despite a ~25% elevation in the resting leg of the trained group. In a recent study by the same authors using a longitudinal design, the attenuation of muscle protein synthesis was observed in the trained leg after exercise in the fed state using the same absolute workload pre and post training (Phillips *et al.*, 2002). An elevation in resting muscle protein synthesis was also seen after training (Phillips *et al.*, 2002). A training-induced attenuation in protein synthesis following exercise has also been reported in resistance-trained compared to untrained rats (Farrell *et al.*, 1999a). In addition, resting muscle protein synthesis rates were elevated following resistance training (Farrell *et al.*, 1999a). The reduction of maximal muscle protein synthesis rates has hypothesized to result in diminishing returns in terms of protein balance as a result of resistance training (Phillips *et al.*, 2002).

1.4. Regulation of Muscle Growth

Skeletal muscle has an intrinsic ability to change its phenotype (i.e. mass and fibre type composition) in response to physical activity. This process involves quantitative and qualitative changes in gene expression, in particular that of the myosin heavy chain isogenes. It has been shown that switching on one subset and repressing another subset of genes can ultimately result in changes in contractile function (Goldspink *et al.*, 1992). This allows for

the tissue to be optimized for power output, rapid movement or fatigue resistance. These contractile characteristics are determined by the type of myosin crossbridge (Goldspink, 2002).

Both muscle mass and muscle phenotype determine muscle contractile performance, as maximum force output is related directly to the cross-sectional area of the fibres and the speed of shortening (Goldspink, 2002). There is an interest in studying the regulation of muscle mass and phenotype concomitantly, to see if these two processes involve similar mechanisms whereby physical signals result in up-regulation of the expression of specific genes. Stretch has been shown to be a powerful stimulant of muscle growth and muscle protein synthesis in animal models (Goldspink, 1999). Stretch combined with electrical stimulation was found to induce very rapid hypertrophy of the tibialis anterior muscle of adult rabbits (Goldspink *et al.*, 1992). Both force generation and stretch are major factors in activating protein synthesis and the combination of these stimuli seem to have an additive effect (Goldspink, 1999; Goldspink, 2002). Of the two stimuli, it seems that stretch is of greater importance to induce hypertrophy in an animal model (Goldspink, 1999). However, in humans, stretch per se may play a lesser role in muscle hypertrophy (Fowles *et al.*, 2000).

1.4.1. Initiation of Protein Synthesis

Protein synthesis involves the translation of mRNA, which is composed of three complex sequences known as peptide chain initiation, elongation and termination. Of the three processes, there has been a focus on peptide chain initiation. Under stressful situations, such as diabetes (Kimball *et al.*, 1994) and exercise (Fluckey *et al.*, 1996; Gautsch *et al.*,

1998; Baar & Esser, 1999, Farrell *et al.*, 1999b), the control of peptide initiation can be rate limiting for the overall process of protein synthesis (Pain, 1996).

In eukaryotes, protein translation can be separated in three phases (Figure 2): (1) association of initiator tRNA (Met-tRNA_f) and 40S ribosomal subunit to form the 43S preinitiation complex; (2) the binding of mRNA to the 43S preinitiation complex, followed by its progression to the correct AUG initiation codon; and (3) the addition of the 60S ribosomal subunit to assemble an 80S ribosome at the initiation codon, prepared to begin translation of the coding sequence (Pain, 1996).

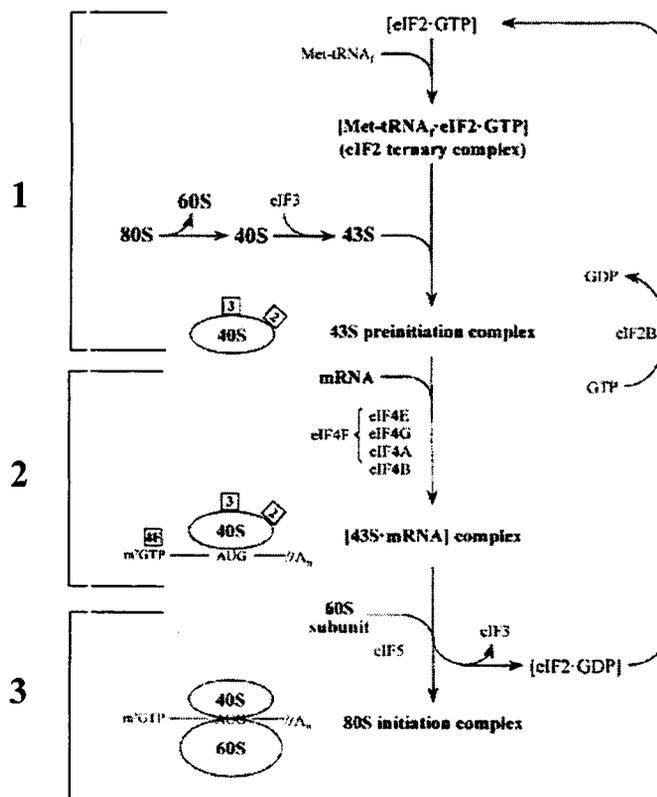


Figure 2. Initiation of protein synthesis. From: Pain (1996).

Three key regulatory factors that play an important role in translation initiation are eIF2, eukaryotic initiation factor 4E binding protein-1 (4E-BP1 also known as PHAS-I) and

p70^{S6k}. The role of the eIF2 system is to facilitate the ribosomal binding of the initiator methionyl-tRNA to the 40S ribosomal subunit, to form the 43S preinitiation complex. The process requires the eIF2 to be in the active GTP-bound state. As a product in the formation of the 80S initiation complex, eIF2 is released in its inactive GDP-bound state. To return to its active state, another GTP must replace GDP. This guanine nucleotide exchange reaction is catalyzed by the eIF2B subunit, which allows for the reuse of eIF2 in ternary complex (Met-tRNA_f · eIF2·GTP) formation. A second point of control during translation initiation is the binding of mRNA to the 43S preinitiation complex, which has been reported to be partially regulated by p70^{S6k} and 4E-BP1. These two factors have been reported to share a similar intermediate signaling pathway that includes the protein kinase mammalian target of rapamycin (mTOR; Anthony *et al.*, 2000). The kinase p70^{S6k} is thought to play an important role in regulating protein synthesis by controlling the translation of many mRNA transcripts that encode components of the translational apparatus. The eIF4F system is composed of eIF4E, eIF4G, and eIF4A. Overall, the eIF4F complex collectively serves to recognize, unfold and guide the binding of mRNA to the 43S preinitiation complex (Gautsch *et al.*, 1998). The availability of eIF4E is critical to this binding and is mediated through its binding with 4E-BP1. In the inactive form (eIF4E bound to 4E-BP1), the eIF4F complex is unable to facilitate the ribosomal binding to mRNA.

The results from a study by Farrell *et al.* (2000) indicated that increases in eIF4E activity are most likely not involved in the stimulation of protein synthesis in skeletal muscle after resistance exercise. In their findings, no significant differences were reported in eIF4E associated with 4E-BP1 between the exercised and sedentary groups. However, the rates of protein synthesis were higher in the exercised group. In a previous study, elevations in

protein synthesis following exercise were consistent with increased eIF2B activity (Farrell *et al.*, 1999b). The combination of these findings would suggest that eIF2B activity is more important to elevations in rates of protein synthesis after resistance exercise than is the eIF4F system.

The activation of p70^{S6k} via phosphorylation was shown to increase following a single bout of high resistance exercise in rats (Baar & Esser, 1999). This increase in p70^{S6k} phosphorylation correlated with skeletal muscle mass gains after six weeks of high resistance exercise using electrical stimulation. In addition, an elevated response of p70^{S6k} activity was reported following stretch in myotubes (Baar *et al.*, 2000). These findings suggest that p70^{S6k} phosphorylation may be an important signal for muscle hypertrophy induced by exercise and stretch and may provide a good intracellular marker for an acute growth stimulus (Baar *et al.*, 2000). These potential mechanisms of protein synthesis have yet to be elucidated in human muscle.

1.4.2. Effects of Insulin on Protein Synthesis

In humans, it has been reported that insulin has the ability to stimulate glucose and amino acid uptake and suppress protein breakdown after exercise (Biolo *et al.*, 1999) in addition to stimulating muscle protein synthesis (Biolo *et al.*, 1995a). Previous studies have reported that a normal increase in rates of protein synthesis measured in situ after acute resistance exercise does not occur when insulin is absent from the medium perfusing the hindlimb of rats (Fluckey *et al.*, 1996). Despite lower circulating insulin concentrations in diabetic rats, increases in muscle mass were observed in response to resistance exercise (Farrell *et al.*, 1999a). However, high intense exercise in combination with low insulin

concentrations found in severely diabetic rats did not produce an anabolic response (Farrell *et al.*, 1998). These findings propose that there is a critical concentration of insulin necessary for muscle protein synthesis to occur (Fedele *et al.*, 2000). Furthermore, it is also implied that insulin may play a permissive role and that there are other factors involved in the regulation of protein synthesis (Farrell *et al.*, 1999a).

The hormone, insulin-like growth factor I (IGF-I), is also secreted in greater amounts as a result of exercise and has been suggested to play a role in protein synthesis (Farrell *et al.*, 1999b). IGF-I was shown to be significantly elevated in moderately diabetic rats, which suggests that IGF-I may facilitate an appropriate anabolic response and compensate for inadequate insulin after resistance exercise (Jacob *et al.*, 1996; Fedele *et al.*, 2001). However, muscle IGF-I in severely diabetic rats was not significantly different as a result of exercise (Fedele *et al.*, 2000). Thus, severe diabetes may hinder a compensatory role for muscle IGF-I under these conditions (Fedele *et al.*, 2000).

1.4.3. Mechano-growth factor (MGF)

It has known that there is local and systemic control of tissue growth. Postnatal growth is believed to be regulated to a large extent by growth hormone produced by the pituitary gland, which causes the release of insulin-like growth factor-I (IGF-I) from the liver. There are a number of cell types that respond to mechanical signals and possess a mechanism for local control of growth, remodeling and repair. It has been recently shown that during intensive exercise most of the circulating IGF-1 is actually derived from the active muscles and is directed toward muscle tissue (Brahm *et al.*, 1997). In skeletal muscle, there are two IGF-1 isoforms expressed, one of which is similar to the main IGF-1 isoform

produced in the liver which has a systemic or endocrine mode of action and another which is apparently designed for an autocrine/paracrine mode of action (Yang *et al.*, 1996). The latter form of IGF-1 has been termed mechano-growth factor (MGF) and is not detectable in muscles unless they are subjected to resistive exercise or stretch (Yang *et al.*, 1996). MGF is derived from the IGF-1 gene by alternative splicing. It is possible that MGF is the end product of mechanotransduction signaling pathways in muscle and other cell types. With respect to skeletal muscle, it has been recognized that there is local control of growth, because if a muscle is exercised, it is only that muscle that undergoes hypertrophy and not all the muscles in the limb. The discovery of MGF provides a link between mechanical stimuli and gene expression, although the nature of the mechanochemical coupling process is not yet known (Goldspink, 1999).

1.4.4. Satellite Cells

Resistance exercise has been reported to cause myofibrillar disruption (Gibala *et al.*, 2000; Stupka *et al.*, 2001; Trappe *et al.*, 2002). The myotrauma induces an acute inflammatory response (Stupka *et al.*, 2001) and initiates the release of a series of growth factors that influence satellite cells to repair the damaged fibres and ultimately contribute to myofibre hypertrophy (Vierck *et al.*, 2000). Satellite cells are myogenic stem cells of mammalian skeletal muscle and are located between the basal lamina and the sarcolemma of myofibres (Vierck *et al.*, 2000). During muscle fibre hypertrophy, cytoplasmic volume occupied by the nucleus is maintained by addition of myonuclei distributed along its length (Kadi *et al.*, 1999; Kadi & Thornell, 2000). Since myonuclei in mature skeletal muscle are post-mitotic and not able to differentiate, satellite cells are considered to be the major source

for the accumulation of myonuclei during postnatal growth (Kadi *et al.*, 1999). There is evidence supporting the role of satellite cells in maintaining the nuclear to cytoplasmic ratio during hypertrophy of muscle fibres as a result of resistance training. In a recent study by Kadi *et al.* (1999), it was reported that women who strength trained for 10 weeks showed a 36% increase in their muscle fibre CSA. The hypertrophy of muscle fibres was accompanied by a substantial increase in myonuclear number, which was positively correlated, to satellite cell number (Kadi *et al.*, 1999). These findings indicate that a muscle with an increased number of myonuclei will contain a correspondingly higher number of satellite cells. It also appears that enlargement of multinucleated muscle cells following 10 weeks of strength training requires the acquisition of additional myonuclei (Kadi & Thornell, 2000). In elite power lifters, the proportion of fibres expressing MHC IIa, the CSA of each fibre and number of myonuclei, satellite cells and fibres expressing markers for early myogenesis were significantly higher compared to the control group (Kadi *et al.*, 1999). It was concluded that the incorporation of satellite cell nuclei into muscle fibres resulted in the maintenance of a constant nuclear to cytoplasmic ratio (Kadi *et al.*, 1999).

1.4.5. Post-transcriptional Regulation of Protein Synthesis

Regulation of protein abundance, at the onset of muscle hypertrophy (acute phase) potentially exists in transcriptional (pretranslational), translational, and/or post-translational mechanisms. It has been proposed that rapid increases in skeletal muscle protein synthesis during overload-induced skeletal muscle hypertrophy in animals and humans is potentially under post-transcriptional regulation (Laurent *et al.*, 1978; Wong & Booth, 1990; Chesley *et al.*, 1992; Yarasheski *et al.*, 1993; MacDougall *et al.*, 1995; Biolo *et al.*, 1995b; Biolo *et al.*,

1997; Phillips *et al.*, 1997; Phillips *et al.*, 1999). In rat gastrocnemius, the unchanged levels of skeletal α -actin mRNA per unit of extractable RNA and per whole muscle after acute exercise suggest that mRNA accumulation is not a major mediator of the acute increase in myofibril protein synthesis rates (Wong & Booth, 1990). Thus, the increase in myofibril protein synthesis after acute isotonic resistance exercise was most likely due to increased RNA activity (protein synthesized per unit of RNA) as opposed to RNA abundance (Wong & Booth, 1990). RNA activity can be considered an index of how efficiently the ribosomal machinery can translate mRNA molecules into protein (Waterlow *et al.*, 1978). In the acute phase, chronic stretch overload induced a rapid and large increase in the mass of the chicken anterior latissimus dorsi muscle, which was disproportional to changes in RNA concentrations (Laurent *et al.*, 1978). In the tibialis anterior of adult muscle, a marked increase in RNA content of muscle was associated with a significant increase in muscle size after two days of stretch and stretch combined with stimulation (Goldspink *et al.*, 1992). The rapid increase in total RNA, which is predominantly ribosomal RNA, indicates that muscle fibre hypertrophy may be controlled primarily at the level of translation and that the rapid increase in the number of ribosomes suggests that more mRNA transcript can be translated into protein (Goldspink, 1999). These findings imply that translational or post-translational regulation and efficiency are responsible for increased protein synthesis at the onset of hypertrophy due to resistance exercise and/or chronic stretch.

In humans, a similar trend of disproportionate increases in protein synthesis and increases total RNA concentrations have been reported (Chesley *et al.*, 1992). Muscle protein synthesis increased in the exercised biceps 4 and 24 h post-exercise; however, total RNA concentration was unchanged whereas RNA activity was significantly elevated

compared with that in the unexercised biceps (Chesley *et al.*, 1992). Chesley *et al.* (1992) concluded that posttranscriptional events are important in mediating acute changes in muscle protein synthesis in response to muscle overload.

While no change in total RNA concentration is indicative of a post-transcriptional mechanism, larger changes in individual mRNAs could occur in the absence of a change in total RNA. Welle *et al.* (1999) examined the effect of resistance exercise on mRNA concentrations encoding the major MHC isoforms and the abundances of total myosin heavy chain mRNA and actin mRNA in adult humans. Supporting the results of Chesley *et al.* (1992), muscle protein synthesis after a series of acute bouts of resistance exercise, was primarily mediated by more efficient translation of mRNA (Welle *et al.*, 1999). The differences in total RNA and MHC mRNA abundances between sedentary and exercised muscles in individual subjects did not correlate significantly with the stimulation of myofibrillar protein synthesis measured ~24 h post-exercise (Welle *et al.*, 1999).

Recently, it has been reported that the up-regulation MHC mRNA expression occurred within 6 h following a single session of heavy-resistance exercise (Willoughby & Nelson, 2002). An increased concentration of a particular mRNA transcript generally leads to an increase in the synthesis of the protein encoded by that mRNA (Welle *et al.*, 1999). In addition, a shift towards a heavy polysome (i.e. increased ribosome associated with mRNA) in muscles 6 h following lengthening contractions suggests the possibility of accelerated translation initiation (Baar & Esser, 1999). These findings suggest that in the acute phase following resistance exercise, an increased rate of translation, possibly in combination with a greater rate of transcription, are responsible for the increase in myofibrillar muscle protein accretion.

1.5. Skeletal Muscle Fibre

The functional units of muscle fibres are the sarcomeres. Each sarcomere contains the myofibrillar proteins myosin (the thick filament) and actin (the thin filament), which are directly involved in muscle contraction (shortening of sarcomeres). In addition to the contractile proteins, myosin and actin, other regulatory proteins (troponin and tropomyosin) are also essential for muscle contraction.

The myosin heavy chain (MHC) contains the myosin heads that interact with actin allowing the muscle to contract. The head region of the MHC contains the actin-binding site of the thin filaments and serves as an enzyme adenosine triphosphatase (ATPase). This enzyme provides the energy required for muscle contraction through the hydrolysis of ATP into adenosine diphosphate (ADP) and inorganic phosphate (P_i). Actin, tropomyosin and troponin make up the thin filament.

Skeletal muscle is composed of different fibre types characterized by specific MHC isoforms, which differ in their ATPase activity (Harridge *et al.*, 1996; Staron, 1997). As the major protein of the thick filament, myosin is currently considered the most suitable marker of fibre type diversity. In addition, fibre types have also been differentiated by their myofibrillar ATPase activity, contractile and metabolic properties. Generally, with respect to contractile properties, MHC isoforms follow an order of shortening velocity: MHC I < MHC IIa < MHC IIx (Hilber *et al.*, 1999), which also corresponds to the order of force/power generating capacity (Bottinelli *et al.*, 1999). In addition, aerobic-oxidative capacity, the hierarchy follows: type I \geq type IIA > type IIX (Bottinelli & Reggiani, 2000).

The major fast isoforms present in limb muscles of small animals are MHC IIB, MHC IID(x), and MHC IIa. They are found in the fast muscle fibre types IIB, IID(X), and

IIA, respectively. Comparative studies on small mammals suggest that increasing body size shifts the profile of the fast MHC isoforms from MHC IIB towards MHC IId(x) and MHC IIa (Hamalainen & Pette, 1995). In other words, fast twitch muscles of larger animals tend to contain less type IIB fibres than does the homologous muscle of smaller animals. This observation corresponds with the finding that humans contain and express transcripts for the MHC IIx isoform, which has led to the reclassification of what was previously identified as MHC IIB isoforms to MHC IIx isoform in humans (Ennion *et al.*, 1995; Pereira Sant'Ana *et al.*, 1997).

Methods such as myofibrillar adenosine triphosphatase (mATPase) histochemistry, immunohistochemistry using antibodies specific to MHC isoforms and electrophoretic analysis of MHC isoforms in single fibres have expanded the understanding of fibre type diversity and their dynamic nature (Pette & Staron, 2000). These various methodologies, combined, have revealed the existence of muscle fibres, which either contain a single MHC isoform (pure fibre types) or two or more MHC isoforms (hybrid fibre types). The hybrid fibre types have been classified as C-fibres. Type IC have a greater ratio of MHC I relative to MHC IIa, type IIC contain an equal ratio of MHC I to MHC IIa, and type IIAC contain a greater ratio of MHC IIa to MHC I (Staron & Hikida, 1992).

Histochemical methods have generally been used to delineate skeletal muscle fibre types. This semi-qualitative classification of skeletal muscle fibre types is based on the pH lability of their mATPase activity. This method has been used in many investigations and has then been modified to show a continuum of fibre types (Pette & Staron, 1990; Staron, 1991; Staron & Hikida, 1992). A variety of staining intensities between the fast fibre types IIA and IIX and fibre types I and IIA suggests a range of fibre types expanding beyond the

three major types (Staron, 1991). It is the coexpression of specific combinations of the major MHC isoforms (MHC I, IIa, and IIx) that results in the formation hybrid fibres. Hybrid fibres bridge the gaps between pure fibre types in the fibre type continuum.

A main limitation of muscle morphology analysis is the ability to accurately delineate pure and hybrid fibres. A more accurate method of classifying single muscle fibres is through MHC isoform separation using MHC sodium dodecyl-polyacrylamide gel electrophoresis (SDS-PAGE). The electrophoretic gel separation is a more quantitative method of determining myosin isoforms, which based on molecular weights of the MHC isoforms (Harridge *et al.*, 1996). This more sensitive method reduces the subjectivity of classifying hybrid fibres through a visual categorization, as is required when histochemically classifying muscle fibres. The misclassification of type IIAX fibres is attributed to varying amounts of MHC IIa and IIx expressed which results in different staining intensities (Staron, 1991). When muscle fibres are histochemically classified and the type IIAX hybrid is not identified, the IIX population maybe overestimated because type IIAX hybrids have been misclassified as type IIX (Kraemer *et al.*, 1996). The proportions of MHC IIa and IIx expressed in type IIAX fibres are difficult to assess histochemically. Such a misclassification may be over represented in trained individuals where many fibres classified as type IIX could contain a small portion of MHC IIa (Staron, 1991).

Previous studies have reported a correlation between histochemically derived fibre types and MHC content in single human muscle fibres (Staron, 1991; Staron & Hikida, 1992; Fry *et al.*, 1994) and entire muscle biopsy samples (Perrie & Bumford, 1986; Adams *et al.*, 1993). The good relationship between the two methods helps to validate the use of routine histochemical techniques to distinguish human skeletal muscle types. Fry *et al.*

(1994) quantitatively analyzed this relationship and found significant correlations (ranged from 0.80 to 0.93), which strengthened the relationship between the percentage fiber type area and the MHC content determined by using histochemical and electrophoretic techniques, respectively.

A combination of histochemical and MHC analysis may be essential for a more accurate assessment of fibre type composition. To a degree, the relationship between fibre type area and MHC content depends on the ability to accurately distinguish skeletal muscle fibre types using histochemical methods. The presence of hybrid fibres, in which two MHCs are coexpressed, may increase the possibility of misclassification using semi-quantitative, subjective criteria (Staron, 1991). An increase in the number of hybrid fibres, due to resistance-training-induced fibre transformations, can cause an under- or over-estimation of the degree of change in fibre type composition (Staron, 1991). The strong correlations between the percentage fibre area and the MHC content increases the validity of using mATPase histochemistry for assessing fibre type composition (Staron, 1991; Fry *et al.*, 1994). However, a major source of variance can be accounted for by the compression of fibre types (type I, IIA, IIX, IC, IIC, IIAC and IIAX) to the three major types in order to match the three MHC isoforms (Fry *et al.*, 1994; Andersen & Aagaard, 2000). This is required because adequate mean area and percentage MHC measurements cannot be satisfactorily determined from the minor type IC, IIC and IIAC fibre type populations (Staron & Hikida, 1992). Type IIX fibres can be histochemically misclassified especially in trained muscle (Staron & Hikida, 1992; Andersen & Schiaffino, 1997). Type IIX fibres may contain both MCH IIa and MCH IIX, and therefore they may not always be a pure type IIX fibre (Staron & Hikida, 1992; Andersen & Schiaffino, 1997).

1.5.1. Skeletal Muscle Fibre Type Transitions

Muscle fibres have the intrinsic ability to change their phenotype under various conditions. The changes in MHC isoforms tend to follow a general scheme of sequential and reversible transitions dependent on the stimulus:



It has been widely reported that resistance training induces a fibre type transition from type IIX to type IIA, which has shown to occur in young and old untrained men and women (Staron *et al.*, 1989; Staron *et al.*, 1991; Adams *et al.*, 1993; Staron *et al.*, 1994; Fry *et al.*, 1994; Andersen *et al.*, 1994b; Bamman *et al.*, 1998; Carroll *et al.*, 1998; Sharman *et al.*, 2001; Williamson *et al.*, 2001). This shift in phenotypic profile has been confirmed with changes in MHC content and mATPase histochemical analysis. Muscle fibre hypertrophy appears to take at least 6-8 weeks to occur, however, the replacement of MHC IIx with MHC IIA was detected, and confirmed by MHC analysis, after as little as two weeks of high intensity resistance training, in women (Staron *et al.*, 1994). Although the most consistent finding as a result of resistance exercise has been a decrease in the type IIX fibre with a reciprocal increase in the type IIA fibre distribution in young adults, only a few studies have been able to report significant changes in both fibre type distributions using either histochemical (Staron *et al.*, 1989; Staron *et al.*, 1991; Andersen *et al.*, 1994b) or electrophoretic (Adams *et al.*, 1993; Williamson *et al.*, 2001) techniques. Discrepancies and non-significant changes (i.e. trends) can be attributed to subjects' initial training status, training protocols, intra-subject variability and misclassification of hybrid fibres.

The training status of subjects prior to participating in resistance training can influence the fibre type transition. After a three month period of strength training, a decrease

in type IIA fibres were observed in male soccer players using traditional mATPase histochemistry analysis (Andersen *et al.*, 1994a). Also, when single fibres were analyzed for MHC isoform content, less than 1% of the fibres contained only MHC Iix, whereas the histochemical ATPase staining revealed 6-10% type IIX fibres (Andersen *et al.*, 1994a). It was concluded that almost all histochemically classified type IIX fibres of athletes are co-expressing MHC isoforms Iia and Iix. Perhaps interval training, in which soccer players are regularly engaged, induced an initial general fibre shift from type IIX to IIA. It is possible that the majority of these “pre-trained” muscles consisted of a larger pool of type IIA fibres from which changes may occur after resistance training.

At least eight weeks of progressive, high-intensity resistance training has been shown to be effective in inducing a fibre shift from type IIX to type IIA in young individuals. A study by Williamson *et al.* (2000) in an older population showed a significant increase in the expression of MHC I as a result of 12 weeks of progressive resistance training involving low-volume, low intensity training using only three sets of leg extensions per workout session. However, in another study conducted by Sharman *et al.* (2001) showed that elderly subjects undergoing heavy resistance training showed a similar shift in the expression of MHC isoforms from MHC Iix to MHC Iia that has been seen to occur in younger subjects. These conflicting findings may be due to lower intensity of the training program used by Williamson *et al.* (2000), which may not have resulted in the recruitment of higher-threshold type IIA and IIX motor units. These findings suggest that high intensity resistance training is the necessary stimulus for a type IIX to IIA fibre transition to take place.

A reduction of hybrid fibres in both young and old populations have been reported to occur with resistance training (Williamson *et al.*, 2000; Williamson *et al.*, 2001).

Williamson *et al.* (2001) reported an increase in pure MHC IIa distribution and a decrease in hybrid (I/IIa, I/IIa/IIx, and IIa/IIx) and MHC IIx proportions. Thus, it appears that alterations in the predominance of MHC isoforms within hybrid fibres contribute to the increase in the MHC IIa proportion (Williamson *et al.*, 2001). Also, electrophoretic analysis of muscle cross-sections showed a significant increase in MHC IIa proportion and subsequent decrease in MHC IIx in young individuals (Williamson *et al.*, 2001). These investigations support the notion that the proportion of hybrid fibres can influence results and it is important to consider these fibre populations to obtain accurate changes within skeletal muscle.

It was first proposed by Goldspink *et al.* (1991) that the gene encoding the fast type MHC IIb might be the default gene that provides a readily available pool of fibres that can transform into type IIA fibres as a response to overload and stretch in animals. Adams *et al.* (1993) published the first study to confirm resistance training-induced alterations in MHC composition of human skeletal muscle. This fibre type conversion from type IIX to IIA may be considered a favourable adaptation because of a greater oxidative capacity, and hence fatigue resistance, of type IIA fibres compared to type IIX (Staron *et al.*, 1984), which would be a benefit in the performance of multiple set, high intensity weight lifting bouts (Staron *et al.*, 1983). It has been suggested that fibres containing the MHC IIa isoform are associated with an metabolic profile (i.e. enzyme profile and a speed of contraction) better suited for strength-demanding muscle performance that involves repeated lifts than fibres containing MHC I or IIx isoforms (Staron *et al.*, 1984). Therefore, it has been suggested that an increase in the expression of MHC IIa isoform, with resistance training, shows an adaptation toward more efficient metabolic properties within the fibre (Sharman *et al.*, 2001).

Type IIx MHC mRNA expression was reported to be the most responsive to a single heavy-resistance exercise session when compared to MHC IIa and MHC I isoform mRNA following (Willoughby & Nelson, 2002). However, with continued resistance training, type IIx MHC gene expression could be down-regulated as muscle fibres can undergo alterations toward the type IIa/IIx and/or type IIa MHC phenotype. It has been reported that most fibres contain either a single mRNA and the corresponding protein (pure fibre) or the coexistence of two mRNAs and MHC isoforms (Andersen & Schiaffino, 1997). Also, a mismatch between MHC mRNA expression and protein expression suggests that phenotypic transition is a relatively slow process, which may account for the differential timing of up- and down-regulation between MHC mRNA and protein. This supports the notion of fibre transition only occurring as a result of long term resistance exercise training.

1.5.2. Skeletal Muscle Fibre Hypertrophy

Heavy resistance training has been shown to be a potent stimulus for muscle fibre hypertrophy to occur in both untrained men and women (Staron *et al.*, 1989; Staron *et al.*, 1991; McCall *et al.*, 1996; Green *et al.*, 1998; Goreham *et al.*, 1999; Andersen & Aagaard, 2000; Widrick *et al.*, 2002). An increase in myofibrillar contractile proteins during elevated muscle protein synthesis after an acute bout of exercise, can ultimately lead to muscle hypertrophy (Welle *et al.*, 1999).

Hypertrophy appears to occur in all three major muscle fibre types (Staron *et al.*, 1989; Staron *et al.*, 1991; Hather *et al.*, 1991), however, the magnitude of increase seems to be fibre type specific with type II fibres showing the largest increase (Staron *et al.*, 1989; Hather *et al.*, 1991). As well, preferential type II fibre hypertrophy has also been shown to

occur (Andersen & Aagaard, 2000) with no significant change in type I muscle fibre size. There have been varying degrees of hypertrophy reported from a modest increase of 10-25% (McCall *et al.*, 1996; Green *et al.*, 1998) extending as high as 57% in type II fibres (Staron *et al.*, 1989). Muscle fibre hypertrophy results in an increased amount of contractile protein and is reflected by a greater cross-sectional area (CSA) of the muscle cell. The differences between the fibre specific hypertrophic responses between studies can be attributed to the different resistance training programs and the duration of the training (Hather *et al.*, 1991; Kraemer *et al.*, 1995).

Most studies measuring muscle fibre hypertrophy have used a single-site muscle biopsy technique and histochemical staining to quantify fibre CSA (Staron *et al.*, 1989; Staron *et al.*, 1991; Staron *et al.*, 1994; McCall *et al.*, 1996; Green *et al.*, 1998; Goreham *et al.*, 1999). It appears that high-resistance training over a training period of at least 6-7 weeks is required to detect an increase in muscle fibre size using histochemical staining methods (Staron *et al.*, 1989; Staron *et al.*, 1991; McCall *et al.*, 1996; Green *et al.*, 1998; Goreham *et al.*, 1999).

Other investigations have used magnetic resonance imaging (MRI) to detect changes in whole muscle CSA (McCall *et al.*, 1996; Conley *et al.*, 1997; Akima *et al.*, 1999). There have been poor correlation between relative changes in muscle CSA measured using MRI and relative changes in muscle fibre CSA measured histochemically from single muscle biopsies (McCall *et al.*, 1996; Narici *et al.*, 1996). McCall *et al.* (1996) reported similar increases in the group's average for mean fibre area (14.5%) and biceps brachii CSA (13.3%). Although the relative increases in the group means may be similar, limitations in the procedures used to obtain the measurements could have introduced variability in the

individual values, resulting in the poor relationship between the two measures of hypertrophy. MRI images contain connective tissue, which could change with resistance training, but does not affect measurements of histochemically derived fibre CSA (Phillips, 2000). The location of the scan site, alignment of the scan, the ability of investigator to obtain a reliable and objective measurement of muscle CSA are additional factors that could increase individual variability measuring hypertrophy with MRI (Phillips, 2000). Also, selective muscle group hypertrophy seen in human quadriceps may have contributed to the poor correlation between increases in fibre CSA and whole muscle hypertrophy (Narici *et al.*, 1996).

With respect to muscle fibre area measurements, the main issue is the appropriateness of single-site biopsy for determination of muscle characteristics, because studies have concluded that human muscle is heterogeneous in fibre composition (Elder *et al.*, 1982; Henriksson-Larsen *et al.*, 1983; Lexell *et al.*, 1983). It is unknown whether fibre areas differ between sites within a muscle, however, inherent variability in the fibre composition of muscle between biopsy sites could influence the calculation of the mean fibre area (McCall *et al.*, 1996). Recently, using sequential estimation analysis, McCall *et al.* (1998) reported a sample of 50 or more fibres would be sufficient to characterize mean type I and II fibre areas from a typical needle muscle biopsy sample. However, in a follow-up study, it was reported that 50 fibres provide an accurate representation of type IIX(B) fibres and 150 and 200 fibres are required to obtain the same accuracy of cross-sectional area of type I and IIA fibres, respectively (McGuigan *et al.*, 2002). Previously, Elder *et al.* (1982) suggested that sampling of three to five biopsies sites would reduce the variability of fibre type distribution between locations within the muscle which would reduce calculated

variables i.e. changes in percent area. Thus, it appears that the number of fibres needed to accurately represent mean fibre cross-sectional area varies between fibre subtype. Therefore, a greater number of fibres obtained from 3-5 biopsy sites (Elder *et al.*, 1982) or repeat biopsies from a single site (Kraemer *et al.*, 1995; McCall *et al.*, 1996) appear necessary to accurately characterize the mean fiber type population and reduce fibre distribution variability.

Skeletal muscle enlargement has been credited primarily to increases in fibre CSA, however, a controversy exists as to whether muscle fibre hyperplasia plays a role (Taylor & Wilkinson, 1986; Antonio & Gonyea, 1993). Although, there is evidence for muscle hyperplasia in some animal models contributing to muscle hypertrophy (Antonio & Gonyea, 1993), there is limited support for this phenomenon in humans.

1.6. Muscle Strength

The basic principle underlying increases in maximal force production for a given movement is through overloading skeletal muscle by repeated force development at levels above those encountered in daily activities (Hellebrandt, 1951). It is clear that muscle strength increases following a progressive high-resistance training program and these have been reported as a voluntary one-repetition maximum (1 RM; McCall *et al.*, 1996; Goreham *et al.*, 1999), voluntary 6 RM (Widrick *et al.*, 2002) or maximal voluntary isokinetic torque production (Higbie *et al.*, 1996; Goreham *et al.*, 1999). In general, muscle strength has been established to be proportional to its cross-sectional area (Sale *et al.*, 1987). However with poor correlations between muscle mass and strength gains, increases in strength after heavy

resistance exercise are suggested to be a result of muscle hypertrophy and/or increased neural activation (Sale, 1988; Higbie *et al.*, 1996; Chilibeck *et al.*, 1998; Widrick *et al.*, 2002).

Higbie *et al.* (1996) reported that after 10 weeks of unilateral concentric only or eccentric only knee extension training muscle hypertrophy was induced by both training protocols. In addition, test mode specificity was observed, such that changes in strength were greatest when measured during the muscle action used in training (i.e. eccentric training increased strength measured with eccentric but not concentric muscle actions and that concentric training increased strength measured with concentric but not eccentric muscle actions). In addition, eccentric training increased strength measured with eccentric muscle actions more than concentric training increased strength with concentric muscle actions (Higbie *et al.*, 1996). It was concluded that muscle hypertrophy and neural adaptations appeared to contribute equally to the changes in strength after both eccentric and concentric training.

During the early phases of resistance training, an increase in muscle strength has not been accompanied by muscle hypertrophy (Staron *et al.*, 1989; Chilibeck *et al.*, 1998; Akima *et al.*, 1999), which has been demonstrated by poor correlations between muscle mass and strength gains (Chilibeck *et al.*, 1998). Early strength gains have been attributed to adaptations within the nervous system such as increased motor neuron activation, improved motor unit synchronization, a reduced bilateral deficit, increased activation of synergistic muscles, or decreased activation of antagonistic muscles (Sale, 1988). Changes in muscle and muscle fibre CSA have not been observed which supports the theory of an increased neural drive playing a predominant role in early strength gains. Phillips (2000) proposed two potential problems with this notion. First, the methodology for measuring muscle and

muscle fibre CSA may lack the sensitivity to detect small but significant increases. Second, it may be that increases in myofibrillar protein density within a fibre could increase before increases in fibre diameter. These proposed hypotheses introduce the possibility that increases in myofibrillar protein content could occur earlier than previously thought (Phillips, 2000). Shifting of MHC isoforms during resistance training has been seen to take place before detectable increases in fibre CSA (Staron *et al.*, 1994). This observation lends support to the notion that early changes in gene expression and protein translation could take place to increase myofibrillar density which may take place in parallel to changes MHC expression.

1.6.1. Unilateral Strength Training

With respect to unilateral training, a cross-training/cross-education effect is a potential adaptation that may occur. This involves changes in the torque-producing capabilities of the untrained limb as a result of unilateral training. This phenomenon has been attributed to neural adaptations in the untrained limb that may occur with training, such as a decreased inhibition and/or increased stimulation of the active motor units, so that a greater flow of impulses diffuses to the untrained limb (Kannus *et al.*, 1992).

Electromyography (EMG) has been used to examine the neural adaptations associated with resistance training. Recent studies investigating unilateral strength training have shown no change in EMG amplitude during training, with increases in peak torque production (Evetovich *et al.*, 2001; Ebersole *et al.*, 2002). These observations suggest that hypertrophic factors, independent of neural adaptations account for strength gains, at least with unilateral training programs (Evetovich *et al.*, 2001; Ebersole *et al.*, 2002). In addition, it has been speculated that muscle hypertrophy occurs earlier when simpler exercises such as

single-joint exercises (arm curl or knee extension) are performed (Chilibeck *et al.*, 1998). Quadriceps CSA as measured by magnetic resonance imaging (MRI) by Higbie *et al.* (1996) increased 5.0 - 6.6% following 10 weeks of unilateral knee extension training. Thus, when simpler exercises performed unilaterally, muscle hypertrophy may play a more dominant role compared to neural mechanisms in strength increases after a prolonged training period.

1.7. Rationale and Statement of Purpose

Resistance exercise is considered a potent stimulus for changes in skeletal muscle protein synthesis. In order for these adaptations to occur, remodeling of muscle protein is required. An acute bout of exercise has been shown to increase muscle protein turnover and affect muscle protein balance (Biolo *et al.*, 1995b; Phillips *et al.*, 1997; Phillips *et al.*, 1999). The synergistic effects of amino acid availability and exercise result in a net positive protein balance, which allows for the accretion of muscle protein (Biolo *et al.*, 1997; Tipton *et al.*, 1999a; Rasmussen *et al.*, 2000; Tipton *et al.*, 2001). In a cross-sectional design using resistance-trained and untrained humans, a reduction in the acute exercise-induced increase in protein turnover was observed in the trained group when exercised at the same relative intensity (Phillips *et al.*, 1999). In a longitudinal bilateral training protocol, a similar training-induced attenuation of human muscle protein synthesis, following an acute bout of exercise at the same absolute workload, has been reported in the fed state (Phillips *et al.*, 2002). A heavy progressive resistance-training program has been shown to induce muscle fibre hypertrophy (Staron *et al.*, 1989; Staron *et al.*, 1991; Hather *et al.*, 1991) and the shifting of fibre type IIX to type IIA (Staron *et al.*, 1989; Adams *et al.*, 1993; Bamman *et al.*, 1998; Williamson *et al.*, 2001). This change in muscle phenotype would require a

significant stimulation of muscle protein synthesis or a reduction of breakdown. In order to maximally stimulate skeletal muscle tissue, progressive training programs have been utilized such that the same relative workload is placed on the training muscle in order to account for strength gains during training. Many studies investigating resistance training-induced adaptations in skeletal muscle have commonly used either a cross-sectional (Phillips *et al.*, 1999) or longitudinal bilateral training design (Phillips *et al.*, 2002). The purpose of this study was to investigate resistance training-induced adaptations in skeletal muscle morphology and mixed muscle protein synthesis at rest and after exercise at the same relative intensity using a longitudinal progressive unilateral leg resistance training program in healthy human males.

1.8. Hypotheses

We propose that 8 weeks of progressive unilateral leg resistance training will induce muscle fibre hypertrophy, a shift in type II fibre profile from type IIX to type IIA and an elevated resting muscle protein synthesis in the trained leg versus the untrained leg. In addition, we expect the response of muscle protein synthesis to be reduced in the trained limb compared to the untrained when both are exercised at the same relative intensity, following training.

Chapter II

Resistance Training-Induced Changes in Human Muscle Protein Synthesis and Fibre Morphology

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2.1. Introduction

Muscle protein turnover involves continual and simultaneous synthesis and degradation of all constituent proteins. During this process of protein recycling, a net positive protein balance occurs when synthesis exceeds breakdown. This net positive protein balance leads to muscle protein gains and hypertrophy. High resistance exercise has been shown to be a potent stimulator of muscle protein turnover (Biolo *et al.*, 1995b; Phillips *et al.*, 1997; Phillips *et al.*, 1999). In the fasted state, resistance exercise improves net protein balance compared to rest, however a negative balance is maintained (Phillips *et al.*, 1997). The additive effects of exercise and amino acid supplementation have been shown to be necessary for a net positive protein balance and protein anabolism (Tipton *et al.*, 1999a).

A common fundamental training principle involves overloading the muscle with an external stimulus, which then results in a corresponding adaptation. Stretch and force generation have been reported to increase muscle mass in animals (Goldspink *et al.*, 1991; Goldspink *et al.*, 1992) and high resistance exercise has been shown to induce muscle fibre hypertrophy in humans (Staron *et al.*, 1989; McCall *et al.*, 1996; Green *et al.*, 1998). In addition, resistance training has been observed to induce a fibre type transition from type IIX to type IIA fibres in humans (Staron *et al.*, 1989). Recently, a reduced stimulation of the muscle protein synthetic response following exercise has been reported as a result of resistance training in humans (Phillips *et al.*, 1999) and in rats (Farrell *et al.*, 1999a).

In order for these muscle adaptations to occur, an up-regulation of particular proteins is required starting with an increased abundance of their respective mRNA transcripts. An elevation of myosin heavy chain (MHC) mRNA expression has been reported to occur in the acute phase (~6 h) following a single session of high intensity

resistance exercise (Willoughby & Nelson, 2002). It has been shown that increases in mixed muscle protein synthesis occur within 3 h, and remain elevated up to 48 h, following resistance exercise (Phillips *et al.*, 1997). The rapid synthetic response has lead authors to hypothesize that muscle protein synthesis is primarily under post-transcriptional control (Chesley *et al.*, 1992; Phillips *et al.*, 1997). However, the recent evidence of increased mRNA expression occurring within 6 h following exercise (Willoughby & Nelson, 2002) suggests protein synthesis may be regulated by a combination of increased gene transcription as well as a greater translational efficiency, at least in the acute situation. The effects of training status on muscle protein synthesis and the underlying mechanisms involved have not been widely investigated.

The purpose of this study was to examine resistance training-induced adaptations in skeletal muscle morphology and mixed muscle protein synthesis using a progressive unilateral leg resistance training program in healthy human males. Specifically, we investigated changes in maximum voluntary isokinetic and 1 repetition maximum (1 RM) leg strength; histochemically derived muscle fibre cross-sectional area and fibre type percent area; fibre type and myosin heavy chain distribution; and resting muscle protein synthesis rates at rest and after exercise performed at the same relative intensity.

2.2. Methods

2.2.1. Subjects

Eight healthy males (mean \pm SD; age: 24.8 ± 4.7 yr, height: 179 ± 12 cm, weight: 74.1 ± 12.6 kg, BMI: 23.2 ± 2.8 kg·m⁻²) who were recreationally active (i.e. no more than two upper body resistance exercise bouts per week) were recruited for the study. Each subject was advised of the purposes of the study and the associated risks. Subjects were required to complete a health questionnaire and gave their written informed consent prior to participation. The Research Ethics Board of the Hamilton Health Sciences Corporation approved the project.

2.2.2. Study Design

The design was a within group (two conditions: rest and exercise), unilateral leg training protocol, which consisted of an eight week progressive resistance training using leg press and knee extension exercises. The leg selected for training was randomized in a counter-balanced manner to account for any strength-based leg dominance.

2.2.3. Resistance Training Protocol

Prior to initiating any testing, subjects had a baseline muscle biopsy taken from the medial portion of the vastus lateralis of the randomly assigned training (T) leg.

Approximately, 2-3 days following the biopsy, subjects attended a familiarization session of the isokinetic strength testing apparatus (Biodex-System 3, Biodex Medical Systems Inc., New York) and underwent a series of preliminary leg strength tests (see Strength

Measurements). All isokinetic strength tests were performed on the Biodex and applied to the untrained (UT) and T leg separately.

Subjects commenced the 8-week progressive resistance training protocol approximately 72 h after their initial muscle biopsy to allow any residual soreness to subside. All training and testing was conducted in the Exercise Metabolism Research Group laboratory. Prior to each training session, subjects warmed up their legs on a cycle ergometer (Monarch Ergomedic 828E) for approximately 3-5 min. The dynamic concentric 1 RM for knee extension (KE) and leg press (LP) exercises on their T leg was determined following the warm-up period and reassessed bi-weekly prior to the first workout session. The 1 RM on the knee extension was tested followed by 1 RM of leg press. A subject's 1 RM was defined as the maximum weight he could lift to full extension and hold for a one second count. A progressive training program was used, such that loading levels were monitored through the training protocol and the 1 RM was reassessed every two weeks to ensure 80% 1 RM was maintained throughout the training period.

In week one, subjects performed two sets of 10 to 12 repetitions at 80% 1 RM of each exercise separated by 2 min of rest between sets, and 3 min of rest between exercises. In the second and third week, three sets of each exercise were performed. In weeks four through eight, four sets were completed of each exercise (three sets 80% 1 RM, 10-12 repetitions and a fourth set to failure). Subjects trained twice per week and were given two full days of rest between workout sessions. Isokinetic strength testing was conducted prior to the first training session and before the first workout session in week 8.

2.2.4. Experimental Infusion Protocol

The experimental infusion protocol is shown schematically in Figure 1. Subjects were studied twice. The first infusion protocol (resting infusion protocol) took place with ~72 h of rest after the last workout session. The second, post-exercise infusion protocol was carried out ~48 h following the end of the resting infusion protocol and ~14 h after an acute bout of exercise. The acute bout of exercise was identical to the last training workout session and was performed by both legs using their respective 1 RM.

At 0600, following an overnight fast, subjects had a 20-gauge catheter inserted into the most prominent vein of each forearm while lying in a supine position, in order to sample venous blood and for a primed, constant infusion of isotopically labeled phenylalanine. The catheters were placed such that arm bending would not occlude the infusion. A background venous blood sample was taken before the initiation of any isotopic infusions. At ~0630, a primed, continuous infusion of L-[*ring*-¹³C₆] phenylalanine (99% enriched) was started and was continued throughout the protocol. The priming dose was 2 $\mu\text{mol}\cdot\text{kg}^{-1}$ and the continuous infusion rate was 0.05 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$. Blood samples were taken throughout the 6 h continuous infusion period ($t = 0, 120, 240, \text{ and } 360 \text{ min}$). Muscle biopsies were obtained from both legs within ~10 min of each other at $t = 120$ and 360 min. Muscle samples were immediately frozen in liquid nitrogen and stored at -80°C until further analysis.

2.2.4.1. Isotope

Isotopes were purchased from Cambridge Isotopes (Andover, MA). Phenylalanine was chosen as the tracer because it is not synthesized by the body or oxidized in the muscle.

The isotope, L-[ring-¹³C₆] phenylalanine (99% enriched), was dissolved in 0.9% saline before infusion and its concentration was determined. All isotopes were filtered through a 0.2- μ m filter before infusion and infused using a calibrated syringe pump (74900 Series, Cole Palmer Instrument Company). The infusion rate was calculated by dividing the total volume of infusate (ml) per body weight by the total infusion period (min). The infusion protocol was designed so that steady state was achieved in both the intramuscular and plasma pools.

2.2.4.2. Blood sampling

All blood samples were collected using a 10cc syringe from the designated blood sampling catheter and transferred into a heparinized evacuated tube (Vacutainer™) at $t = 0$, 120, 240, and 360 min for determination of tracer to tracee ratio. The catheter was flushed with 0.9% saline after each sample to prevent clotting. Blood samples were immediately centrifuged at 4°C for 10 min at 4500 rpm. Plasma samples were stored at -80°C until further analysis.

2.2.4.3. Muscle Biopsies

All needle biopsies were obtained from each subject under local anesthesia, using a mixture of 9 parts of 1% lidocaine with epinephrine, and 2% lidocaine and 1 part of 8.5% sodium bicarbonate. After the injection of the anesthetic, an incision was made in the vastus lateralis ~20 cm above the knee. A 5 mm Bergstöröm biopsy needle modified for manual suction was advanced 3-5 cm through the fascia deep into the muscle, with the cutting window closed. With suction applied, the cutting cylinder was opened and closed twice. A sample of ~50-70 mg of mixed muscle tissue was obtained from each biopsy.

The baseline, pre-training muscle sample was placed in optimal cutting temperature (OCT, Tissue Tek[®], Sákura Finetechnical Co. Ltd., Tokyo, Japan) embedding compound and arranged with the fibres perpendicular to the plane in which it was to be cut. The sample was then immediately frozen in isopentane cooled by liquid nitrogen and stored at -80°C until subsequent analysis. The first biopsy ($t = 120$ min) of the T leg during the resting infusion protocol was separated into two portions. One portion was prepared in OCT in the same manner described above and the second was immediately frozen in liquid nitrogen and stored at -80°C until further analysis. The remaining muscle samples taken from the UT leg ($t = 120$ min) and from both T and UT legs ($t = 360$ min) were immediately frozen in liquid nitrogen and stored at -80°C until further analysis.

2.2.5. Isokinetic Strength Measurements

To observe the time course for changes in peak torque production, subjects completed an isokinetic knee extension strength test at 0.52 rad/s on each leg before and after training. During the familiarization session, subjects had all seat settings and dynamometer customized to fit them in a comfortable position. A range of motion resulting of 1.74 rad leg extension was chosen so that the muscle would be exercised over a greater length. Subjects were motivated to give maximal effort through verbal encouragement and visual feedback of their force production throughout the exercise movement. Subjects performed strength tests on the T leg first to allow for extra rest, while the UT leg was undergoing strength tests, before engaging in resistance training.

2.2.5.1. Concentric Torque

Concentric peak torque production was recorded at 0.52 rad/s. The subjects started with their customized seat settings and with their knee aligned with the axis of rotation of the dynamometer lever arm and leg flexed (~1.04 rad). The subjects then extended their leg and exerted a maximal voluntary force (MVC) against the resistance provided by the lever arm, which was strapped in place above their ankle. At the end of the concentric motion (2.79 rad flexion), they were able to relax and flex their leg back to the original position. Subjects then repeated the kicking motion for three continuous repetitions. To monitor strength throughout the training protocol, the peak torque (Nm) was recorded for each of the T and UT legs. The peak torque produced was recorded for all 3 repetitions with the highest value being considered the MVC torque.

2.2.6. Analysis

2.2.6.1. Muscle Fibre Type & Cross-sectional Area

The frozen OCT mounted muscle samples were serially cross-sectioned 10 μm thick on a Microtome cryostat (Model HM5000M, MICROM International, Waldorf, Germany) for histochemical analysis. Tissue samples were adhered to glass slides and allowed to dry overnight at 4°C. Myofibrillar adenosine triphosphate (mATPase) histochemistry was performed using preincubation pH value 4.60 (Brooke & Kaiser, 1970) to determine muscle fibre type composition. Samples were then incubated for 6.5 min in an acidic preincubation solution (50 mM potassium acetate, 17.5 mM calcium chloride) adjusted to pH 4.60 using glacial acetic acid. Slides were then rinsed three times with distilled water. Then the slides were incubated in 3 mM ATP using an alkaline solution (75 mM glycine, 40.5 mM calcium

chloride, 75 mM NaCl, 67.5 mM NaOH, adjusted to pH 9.4) for 45 min at 37°C and agitated at regular intervals in a temperature controlled incubator shaker (G24 Environmental Incubator Shaker, New Brunswick Scientific Co., New Brunswick, NJ). Following the ATP incubation, a rinse with distilled water was done twice and incubated in 1% CaCl₂·2H₂O for 3 min at room temperature. Slides were washed five times with distilled water and incubated in 2% CoCl₂·6H₂O for 3 min at room temperature. Five rinses with distilled water and an incubation in 1% ammonium sulphide for 1 min at room temperature followed. Samples were rinsed with distilled water five times before being dehydrated by incubating for 2 min in each ethanol concentrations (70, 80, 90, 95 and 100%). Samples were then cleared twice in two separate dishes of fresh xylene. After slides were completely air-dried, coverslips were mounted using Permount (Fisher SP15) and allowed to dry overnight.

Sections were viewed under a light microscope (Olympus, BX-60, Olympus America Inc., Melville, NY), images were digitized using a SPOT camera (Model: SP401-115, SPOT Diagnostic Instruments Inc., Michigan, USA) and analyzed using SPOT software (V3.2.4 for Windows, SPOT Diagnostic Instruments, Inc.), ImageJ software (<http://rsb.info.nih.gov/ij/> v1.26, National Institute of Mental Health, MD, USA) and Image Pro Plus (V4.0 for Windows, Media Cybernetics, Silver Spring, MD). The number of images taken at 200x magnification of each sample were between two and three, and largely dependent on the quality of the serial sections. Each image contained approximately 30-50 fibres with a total of 90-150 fibres per sample. Three fibre types (I, IIA and IIB) were distinguished using the ImageJ software by setting cut-off limits according to the darkest, lightest, and intermediately stained fibres. The classification of fibre type is thus, dependent on the intensity of the staining by the mATPase histochemical protocol. At pH 4.60, the

light, intermediate, and dark fibres correspond to fibre type IIA, type IIB and type I, respectively. Sample images were converted to 8-bit, 256 grayscale images, which linearly scale each pixel and assigns a value from between 0 (black) to 255 (white). By setting lower and upper threshold values (0-95 for dark areas, 100-175 for intermediate areas, 180-255 for light areas), the three fibre types were more objectively classified. Direct tracings using the Image Pro Plus software determined fibre cross-sectional areas, which were expressed in μm^2 . Fibre type percent area and fibre type distribution measurements were also calculated and expressed.

2.2.6.2. Myosin Heavy Chain Analysis

MHC analysis was performed on all the biopsy samples using sodium dodecyl sulfate (SDS)-polyacrylamide electrophoretic techniques and the following protocol was taken from Staron *et al.*, 2000. Four to six serial cross-sections (20 μm thick) from each biopsy were placed in 500 μl of a lysing buffer containing 10% (w/v) glycerol, 5% (v/v) 2-mercaptoethanol, and 2.3% (w/v) SDS in 62.5 mM Tris (hydroxymethyl) aminomethane HCl buffer (pH 6.8) and were heated for 10 min at 60°C. Small amounts of the extracts (3-5 μl) were loaded on 4–8% gradient SDS-polyacrylamide gels with 4% stacking gels, run overnight (19–21 hr) at 120 V, and stained with Coomassie Blue. MHC isoforms were identified according to their apparent molecular masses compared with those of marker proteins and migration patterns from single fiber analyses. Relative MHC isoform content was subsequently determined using a laser densitometer.

2.2.6.3. Blood Plasma and Muscle Analyses

For determination of amino acid enrichment, blood plasma samples were precipitated free of protein using 0.6 M perchloric acid (PCA). Samples were then centrifuged at 15 000 rpm for 2 min at 4°C. To neutralize the supernatant, 1.25 M potassium bicarbonate (KHCO₃) was added and the reaction was allowed to proceed for 10min, on ice. Samples were centrifuged at 15 000 g for 2 min at 4°C. The supernatant was transferred into a threaded Pyrex[®] tube for derivatization. To determine the enrichment of infused phenylalanine in blood plasma, the *tert*-butyl dimethylsilyl (*t*-BMDS) derivative was prepared. The supernatant from the precipitation protocol was lyophilized in a Speed-Vac rotary evaporator (SC210A-120 Savant Instruments, Farmingdale, NY). To the dried sample, 50 µl of acetonitrile (HPLC grade) and 50 µl *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluoro-acetamide + 1% *tert*-butyldimethylchlorosilane (MTBSTFA + 1% TBDMCS; Pierce Chemical, Rockford, IL) were added, and the sample was heated for 1 h at 90°C. The *t*-BMDS derivative of phenylalanine was then analyzed using electron-impact ionization capillary gas chromatography-mass spectrometry (GC-MS; Models 6890 GC and 5973 MS, Hewlett-Packard) and selected ion monitoring of mass-to-charge ratio (*m/z*) 234, and 240, for the *m*+0, and *m*+6 ions, respectively. The conditions of the GC column (model no. J&W DB-5, 325°C max; Dimension: 15.0 m x 250 µm x 0.25 µm 30 m) consisted of an initial oven ramp temperature of 80°C, held for 2 min; first ramp to 230°C at 20°C/min, held for 2 min; second ramp to 300°C at 50°C/min, held for 2 min; and a post-run at 300°C for 2 min. The split ratio and split flow used were 3.0:1 and 3.6 ml/min, respectively.

Approximately 20-30 mg of wet muscle tissue was analyzed for mixed-muscle protein-bound and free intracellular enrichment of L-[*ring*-¹³C₆] phenylalanine. Muscle

samples were lyophilized to dryness while being incubated on dry ice, overnight. Samples were manually powdered to which 500 μ l 0.6 M PCA was added. After gentle shaking, the samples sat on ice for 10 min and were then centrifuged at 4500 rpm for 2 min. The supernatant was collected and processed in the same manner as plasma samples. The remaining pellet of muscle tissue was washed in 1 ml of distilled deionized water, followed by second wash in 1 ml of absolute ethanol then placed in a rotary evaporator and dried overnight. The dried pellet was placed in 6 N HCl and hydrolyzed for 24 h at 100°C. The protein hydrolysate was passed over ion exchange columns for amino acid isolation/purification to determine protein bound phenylalanine enrichments. The acid hydrolysate was passed over an acid-washed cation exchange column (Dowex Analytical Grade 50W-X8, 100-200 mesh hydrogen form, BioRad Labs, Hercules, CA). Desorption of amino acids was accomplished using one head volume (~5 ml) of 4 N NH_4OH and the eluate from the columns was collected immediately. Samples were then dried using a rotary evaporator. The dried pellet was then derivatized in the same manner as blood plasma samples.

A standard curve was used to calculate the protein-bound (m+6) to (m+0) ratios. A series of standard tracer to tracee ratios (0%, 0.0025%, 0.005%, 0.01%, 0.05% and 0.1%) were prepared and diluted 10 fold. A number of standard quantities (25, 50 and 250 μ L) were dried and derivatized to account for a concentration dependency within a standard curve (Patterson *et al.*, 1998). The standard curve was constructed by plotting the (m+6) to (m+4) ratio against the (m+6) to (m+0). The (m+0) abundance from each hydrolysate sample determined which standard curve was used to convert the protein-bound (m+6) to (m+4) ratio to the (m+6) to (m+0) ratio.

2.2.6.4. Calculations

Fractional synthesis rate (FSR) was calculated from the determination of the rate of tracer incorporation into muscle protein and using the muscle intracellular free phenylalanine enrichment as a precursor, according to the equation:

$$\text{FSR (\%/h)} = \{(Et_1 - Et_0) / [E_p \cdot (t_1 - t_0)]\} \cdot 100$$

where Et_0 is the enrichment in the protein-bound phenylalanine tracer from the first biopsy at $t = 120$ min, Et_1 is the enrichment of the protein-bound phenylalanine tracer from the second biopsy $t = 360$ min, $(t_1 - t_0)$ is the incorporation time (~4 h); and E_p is the mean intracellular ($t = 120$ and 360 min) [$^{13}\text{C}_6$] phenylalanine enrichment during the time period for determination of protein incorporation.

2.2.7. Statistics

The 1 RM measurements were analyzed using a one-way repeated measures and isokinetic strength measurements were analyzed using a two-way repeated measures analysis of variance (ANOVA), with time (1 RM: weeks, isokinetic: pre and post training) and training (UT and T) as within-subject factors. Mixed muscle protein FSR was also analyzed using a two-way within-subject with condition (rest and exercise) and training (UT and T) as within-subject factors. Fibre cross-sectional area, percent area and distribution were analyzed using one-way ANOVA separated by fibre types with condition (UT, pre and post training) as within-subject factors. Wherever ANOVA revealed significant differences ($P <$

0.05), a Tukey post-hoc procedure was used to identify the difference. All data are expressed as means \pm SE.

2.3. Results

2.3.1. Strength Measurements

Prior to training, there was no difference in isokinetic leg strength between legs when tested at 0.52 rad/s concentrically (UT vs. T: $4 \pm 3\%$; $P = 0.55$; Fig. 4). After training, isokinetic strength increased when tested at 0.52 rad/s concentrically ($14 \pm 6\%$; $P < 0.05$; Fig. 4) in the trained leg and with no increase in the contralateral UT leg.

After 4 weeks of training, 1 RM unilateral leg strength increased (KE = $52 \pm 16\%$; $P < 0.01$; Fig. 5A, LP = $38 \pm 9\%$; $P < 0.01$; Fig. 5B). At the end of eighth week, all subjects continued to increase their 1 RM unilateral leg strength compared to rest (KE = $95 \pm 20\%$; $P < 0.01$; Fig. 5A, LP = $80 \pm 17\%$; $P < 0.01$; Fig. 5B) and mid-testing values (KE = $30 \pm 7\%$; Fig. 5A; $P < 0.01$, LP = $29 \pm 4\%$; $P < 0.01$; Fig. 5B).

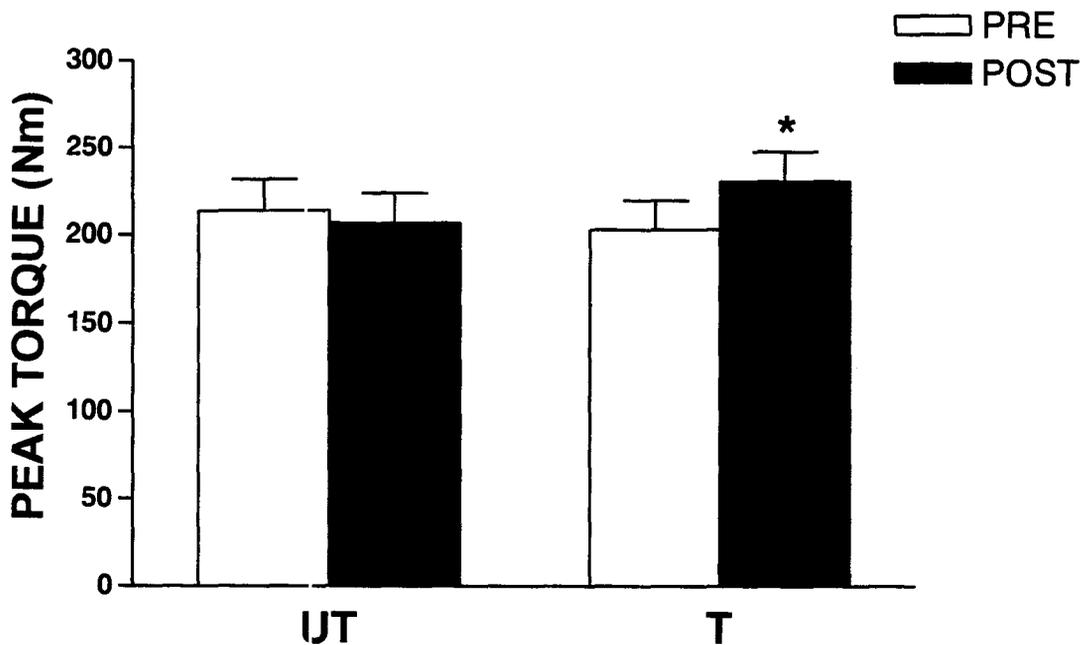
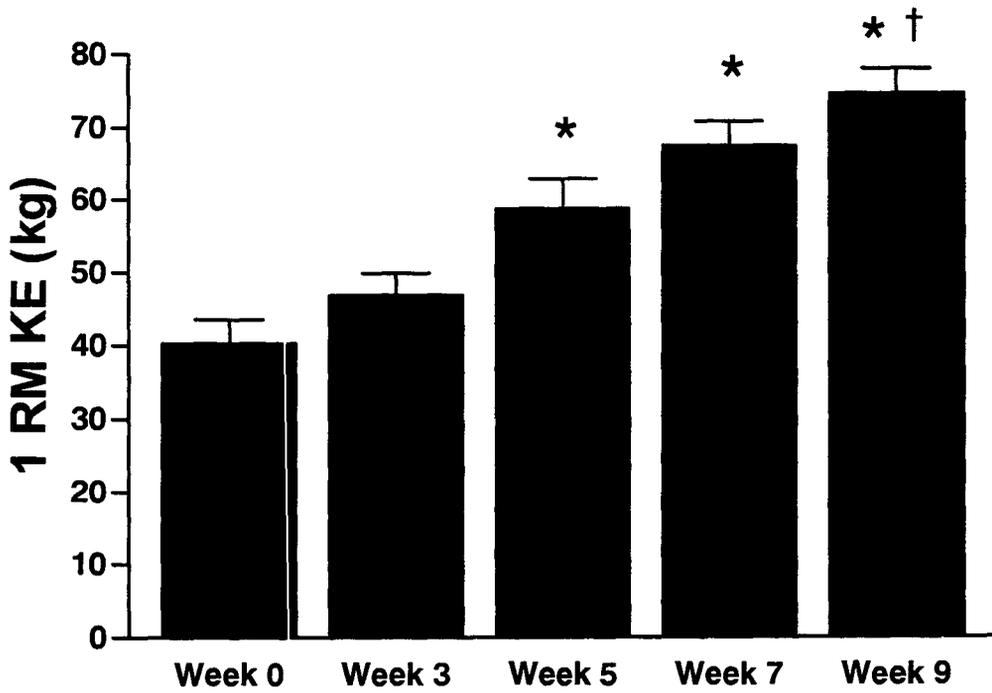


Figure 4. Isokinetic leg strength tested concentrically at 0.52 rad/s. * Significantly different from PRE-T and POST-UT ($P < 0.05$). Values are means \pm SE.

A



B

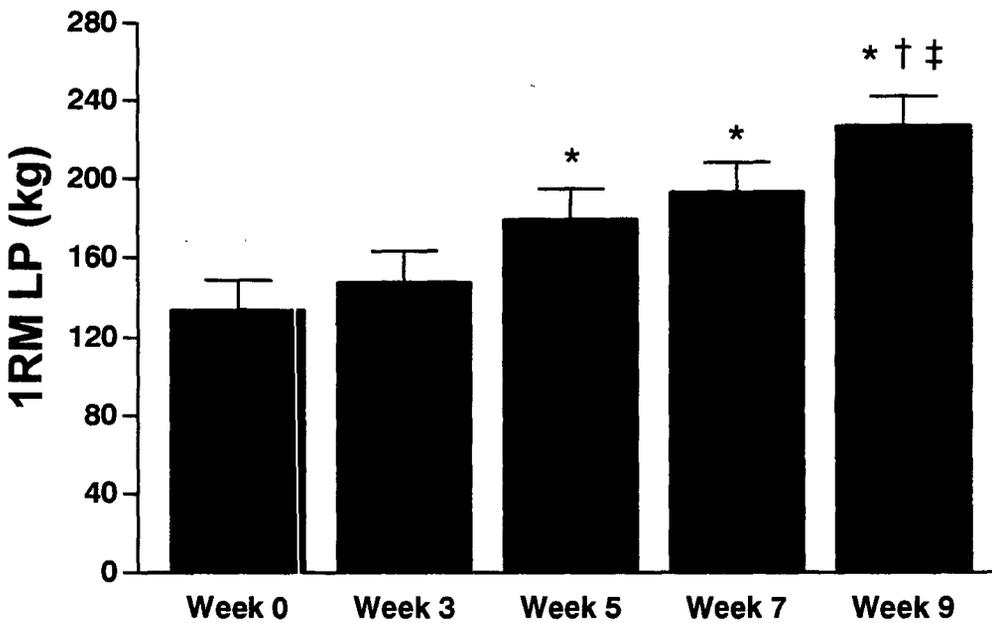


Figure 5. Bi-weekly 1 RM assessments. A) 1 RM knee extension; B) 1 RM leg press. * Significantly different from Week 0 and Week 3 ($P < 0.01$); † Significantly different from Week 5 ($P < 0.01$); ‡ Significantly different from Week 7 ($P < 0.01$). Values are means \pm SE.

2.3.2. Muscle Fibre Type Analysis

The combined mean fibre cross-sectional area of all three major fibre types increased after training ($28 \pm 6\%$; $P < 0.01$). Individually, type I fibre CSA increased ($10 \pm 11\%$; $P = 0.34$; Fig. 6) and significant hypertrophy was seen in type IIA ($32 \pm 8\%$; $P < 0.05$; Fig. 6) and type IIX fibres ($43 \pm 10\%$; $P < 0.05$; Fig. 6) as result of training.

There were no significant increases in the relative percentage of type I ($4 \pm 17\%$; $P = 0.75$; Fig. 7) and IIA fibres ($23 \pm 16\%$; $P = 0.64$; Fig. 7) or decreases in type IIX fibres ($-25 \pm 11\%$; $P = 0.21$; Fig. 7).

There were no significant changes to all three fibre type distribution as a result of training (Fig. 8: type I: $15 \pm 16\%$; $P = 0.68$, type IIA: $16 \pm 15\%$; $P = 0.11$, type IIX: $-30 \pm 11\%$; $P = 0.23$).

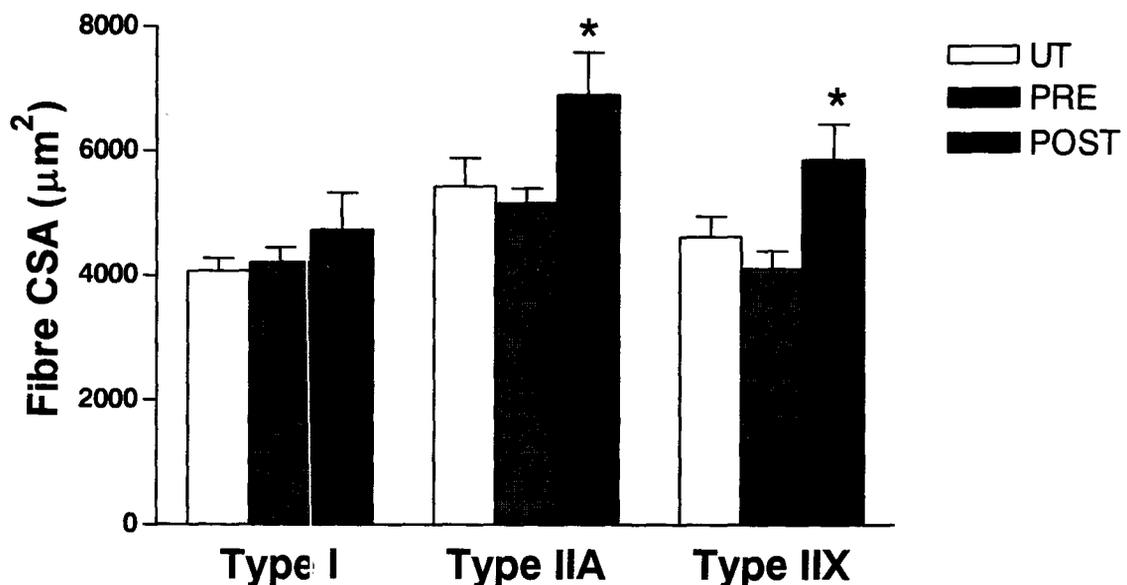


Figure 6. Mean fibre cross-sectional area. *Significantly different from PRE and UT ($P < 0.05$). Values are means \pm SE.

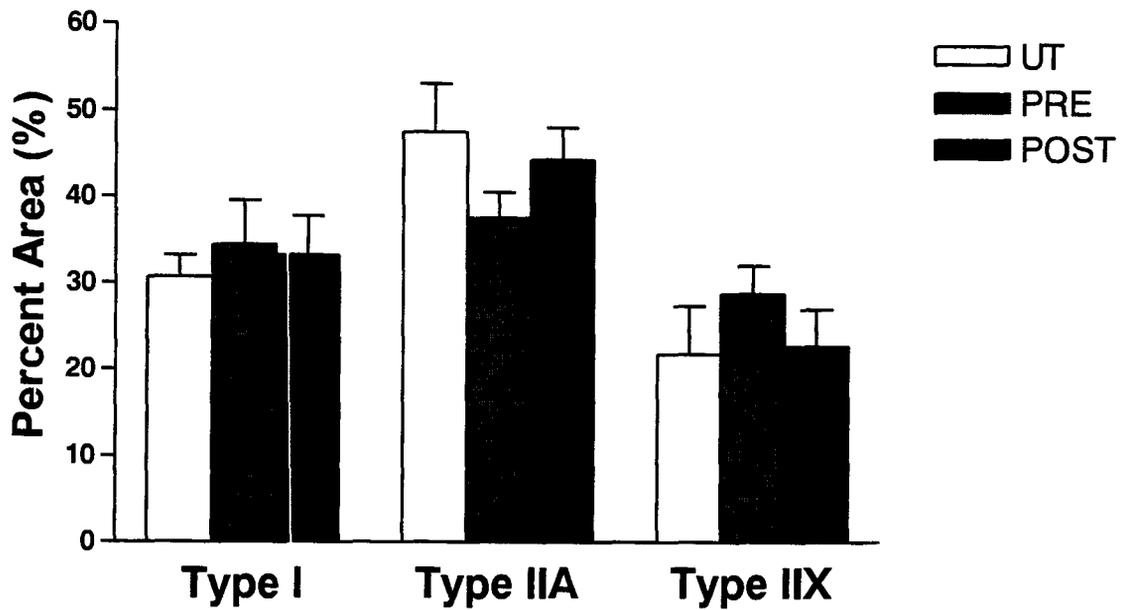


Figure 7. Percent area. Values are means \pm SE.

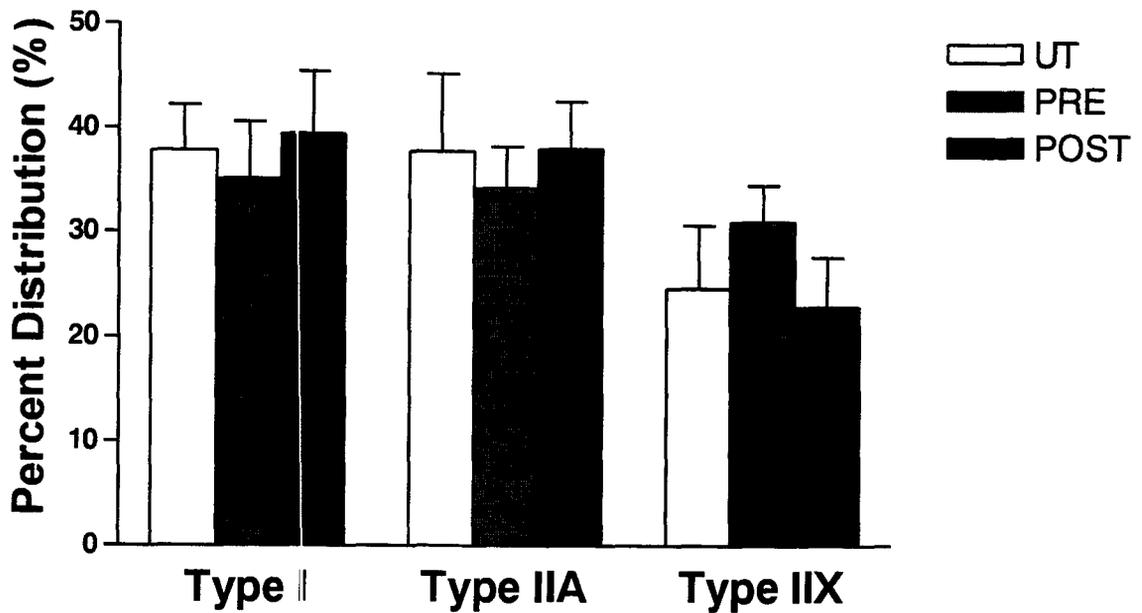


Figure 8. Percent distribution. Values are means \pm SE.

2.3.3. Myosin Heavy Chain Analysis

Changes in MHC isoform content paralleled the histochemically derived percent area changes. Results are shown in Fig. 9. MHC I increased by $2.1\% \pm 8.1\%$ ($P = 0.16$), MHC IIa increased by $16 \pm 3.4\%$ ($P = 0.30$) and MHC IIx decreased by $15.4 \pm 6.2\%$ ($P = 0.17$).

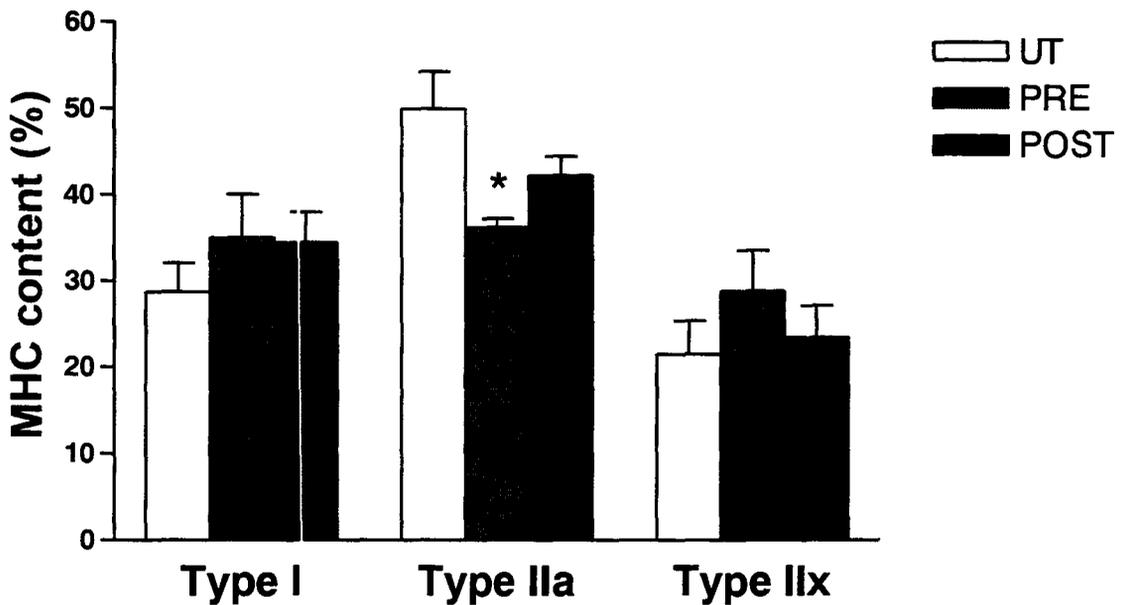


Figure 9. MHC content. Values are means \pm SE. *Significantly different from UT condition.

2.2.4. Myosin Heavy Chain Content and Percent Fibre Area Correlation Analysis

Type I:MHC I	0.84
Type IIA:MHC IIa	0.79
Type IIX:MHC IIx	0.87
Pre	0.81
Post	0.87
UT	0.95
Overall	0.89

Table 1. All values given are significant correlation coefficients ($P < 0.05$). Type I:MHC I, percentage fibre type area I vs. percentage MHC I; Type IIA:MHC IIa, Percentage fibre type area IIA vs. percentage MHC IIa; Type IIX:MHC IIx, percentage fibre type area IIX vs. percentage MHC IIx; pre, post, UT and overall, all percentage fibre type area and percentage MHC values for their respective groups.

The calculated percentage area of the three histochemically determined major fibre types was compared to the MHC content in each biopsy sample. Significant correlations were found in each condition ($P < 0.05$; Table 1).

2.2.5. Mixed Muscle Protein Fractional Synthesis Rate

The mixed muscle FSR response was increased after an acute bout of exercise in the UT leg ($172 \pm 52\%$; $P < 0.01$; Fig. 10) but remained unchanged in the T leg ($28 \pm 18\%$; $P = 0.08$; Fig. 10). An elevated resting FSR was seen in the T leg compared to the UT leg ($76 \pm 32\%$; $P < 0.01$; Fig. 10). The synthetic response in the UT leg was significantly greater ($29 \pm 9\%$; $P < 0.01$; Fig. 10) compared to the T leg, after exercise.

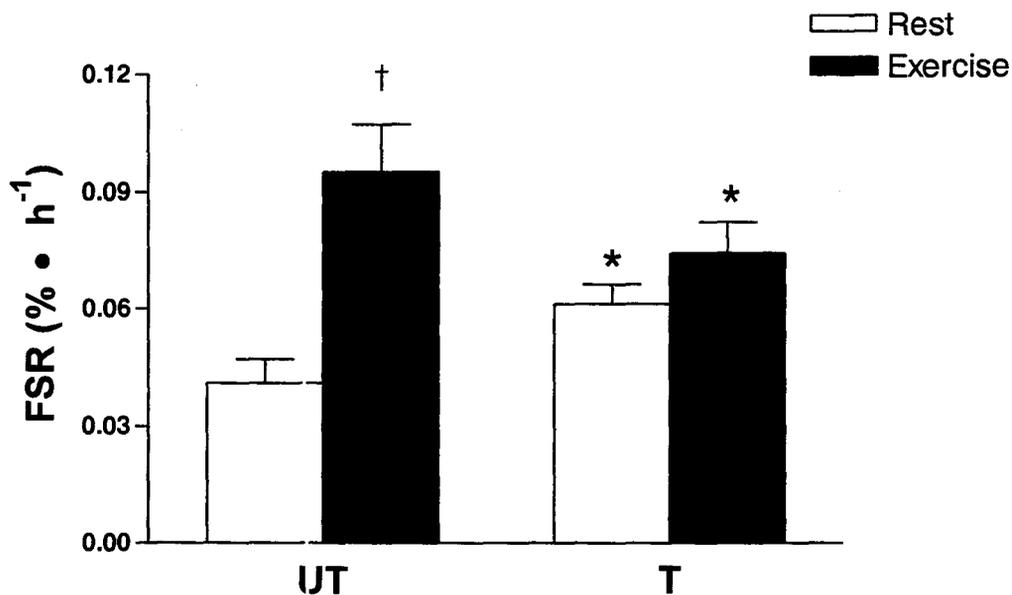


Figure 10. Mixed muscle FSR. *Significantly different from UT within same condition ($P < 0.01$); † Significantly different from all other conditions. Values are means + SE.

Exercise induced muscle protein synthesis was accomplished by each leg completing four sets of 12 repetitions of 80% 1 RM of knee extension (UT: 46.5 ± 2.1 kg; T: 59.9 ± 2.6 kg) and leg press (UT: 144.0 ± 14.4 kg; T: 181.0 ± 11.9 kg) exercises.

2.2.5.1. Blood

Subjects' mean blood $^{13}\text{C}_6$ -phenylalanine enrichment was maintained throughout the both infusion protocols (rest and after exercise; Fig. 11). There was no difference in enrichment during the time points ($t = 120$ and 360 min) when muscle biopsies were taken.

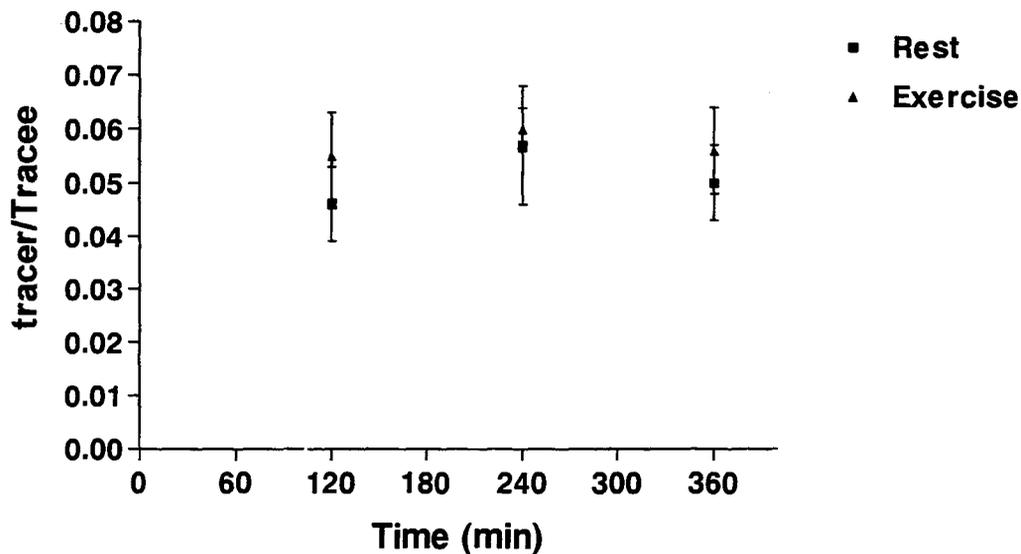
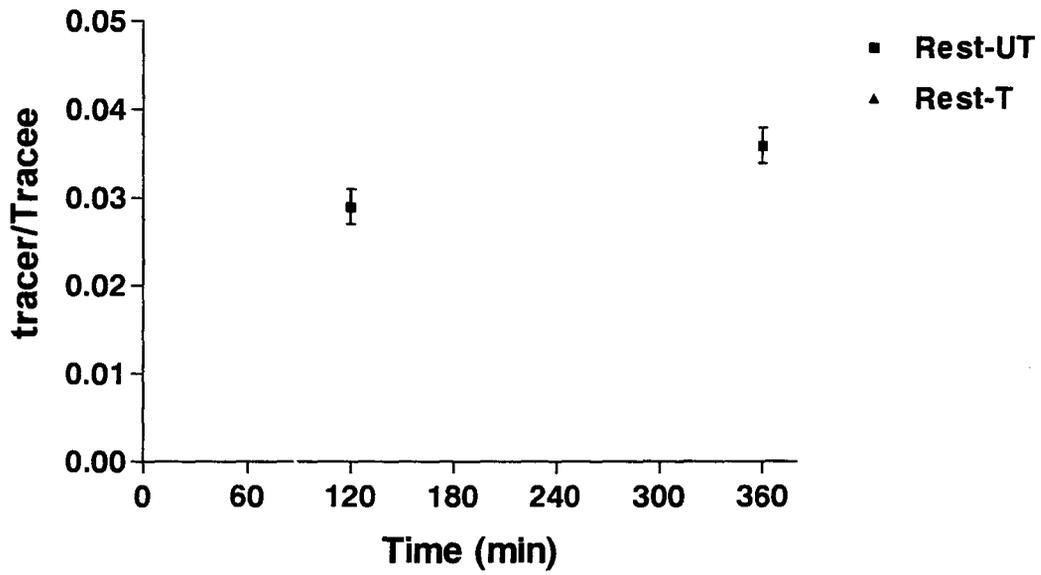


Figure 11. Blood $^{13}\text{C}_6$ -phenylalanine enrichment of individuals during resting and after exercise infusions.

2.2.5.2. Muscle

Mean muscle intracellular $^{13}\text{C}_6$ -phenylalanine enrichment were lower than blood enrichments. Dilution by protein breakdown most likely accounted for the lower values (Biolo *et al.*, 1995b). There was an elevation in enrichment over time for both the T and UT legs. The translated protein-bound enrichments under the resting and post-exercise conditions for both the T and UT legs show an increase in enrichment over the two sampling time points.

A



B

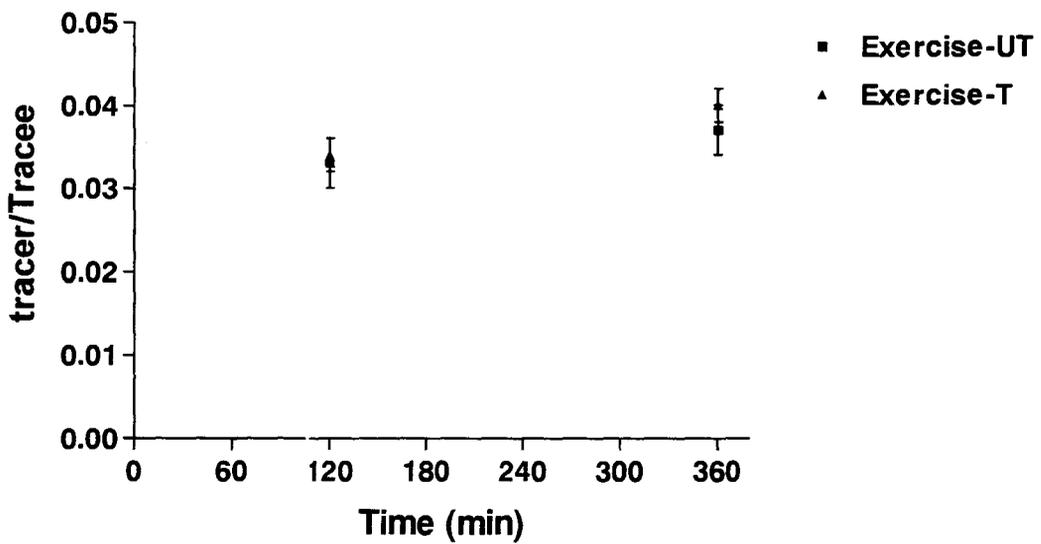
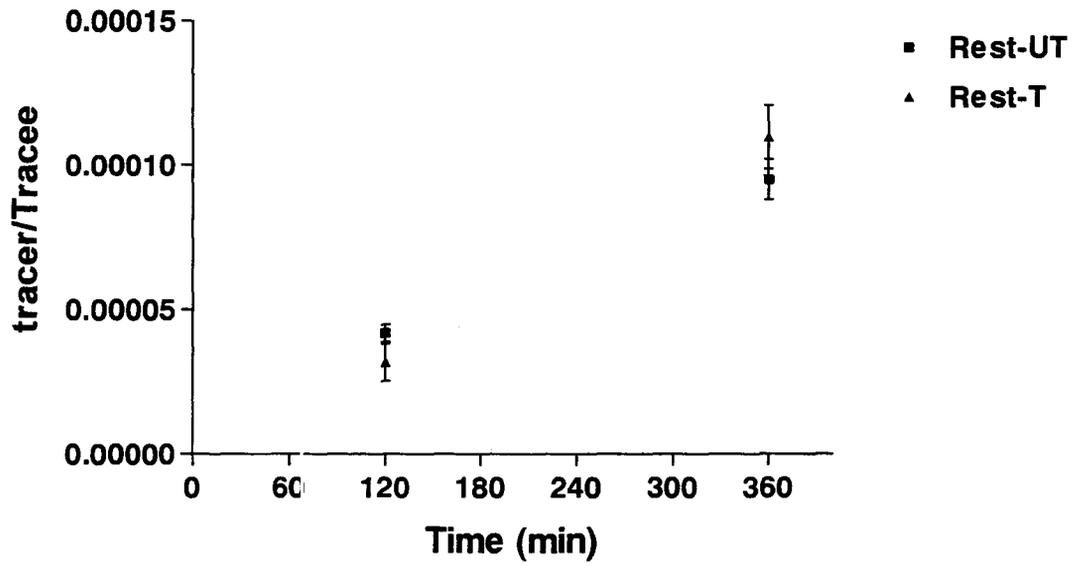


Figure 12. Mean intramuscular $^{13}\text{C}_6$ -phenylalanine enrichment during A) resting infusion and B) infusion after exercise.

A



B

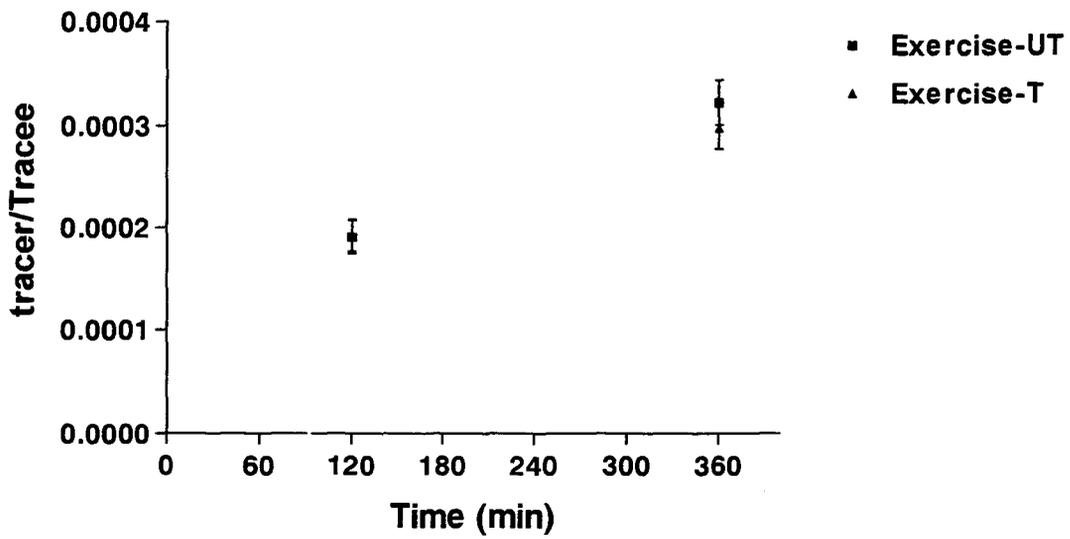


Figure 13. Mean protein bound $^{13}\text{C}_6$ -phenylalanine enrichment during A) resting infusion and B) infusion after exercise.

2.4 Discussion

Mixed muscle protein FSR. The primary novel findings from the present study were an elevated basal muscle protein FSR and a reduced exercise-stimulated increase in FSR in the T leg compared to the UT leg. The basal synthesis rates were measured approximately 72 h after the last training session to avoid residual synthetic response from the final training session. The response to exercise was acutely increased in the untrained leg, whereas there was no significant difference found in the trained leg compared to the respective resting values. Hence, 8 weeks of progressive resistance training attenuated muscle protein synthesis after an isolated bout of exercise at the same relative workload and also resulted in an elevated resting FSR compared to that of an untrained leg.

The heightened exercise-induced muscle protein synthesis response in the UT leg is consistent with previous findings (Chesley *et al.*, 1992; Yarasheski *et al.*, 1993; Biolo *et al.*, 1995b; Phillips *et al.*, 1997; Phillips *et al.*, 1999). In untrained individuals, muscle protein turnover increased 3 h post-exercise and remained elevated over a 48 h period (Phillips *et al.*, 1997). In trained subjects, mixed muscle protein FSR remained elevated at 24 h and returned to baseline at 36 h post-exercise (MacDougall *et al.*, 1995). The shorter time course of elevated muscle protein synthesis reported by MacDougall *et al.* (1995) could be attributed to the trained status of the subjects. In this study, muscle protein FSR was measured ~16 h post-exercise during which the FSR response in the untrained leg was significantly greater than the trained leg. Other studies have reported a training-induced reduction in the muscle protein synthetic response in humans (Phillips *et al.*, 1999; Phillips *et al.*, 2002) and in rats (Farrell *et al.*, 1999a). In a cross-sectional study, this exercise-induced reduction in protein synthesis was also accompanied by attenuation in protein breakdown in highly trained

individuals compared to the untrained group (Phillips *et al.*, 1999). Thus, protein turnover was reduced as a result of training (Phillips *et al.*, 1999). According to our data, a training-induced attenuation of the post-exercise muscle protein synthetic response with an elevated basal FSR, which is also observed in the fed state (Phillips *et al.* 2002), may result in smaller gains in muscle protein accretion as training progresses. From a metabolic standpoint, this adaptation may be due to a trade-off between the desirability of having a greater cross-sectional area of muscle and the energy cost of carrying that extra mass (Goldspink, 2002). Furthermore, a likely adaptation to repeated resistance exercise would be a reduction in exercise-induced muscle damage. It is possible, given the strong association between muscle protein synthesis and breakdown (Biolo *et al.*, 1995b; Phillips *et al.*, 1997; Phillips *et al.*, 1999), that reduced physical damage resulted in a lesser stimulus for a rise in synthesis, however, this hypothesis remains to be tested. Thus, lesser gains (reduced net protein balance) would be attained as skeletal muscle adapts with greater muscle hypertrophy and more efficient metabolic properties.

Muscle fibre morphology. In order to account for the observed muscle fibre hypertrophy, an average net positive muscle protein balance must have occurred over the training period. It appears that feeding soon after each training session was sufficient to allow for muscle protein accretion, as muscle protein synthesis rates are elevated up to 48 h post-exercise (Phillips *et al.*, 1997). Thus, the additive effect of amino acid provision through a regular diet combined with exercise bouts provided a net positive muscle protein balance (Tipton *et al.*, 1999a; Rasmussen *et al.*, 2000; Tipton *et al.*, 2001). More specifically, muscle fibre hypertrophy requires an increase in myofibrillar protein synthesis. Measuring the fractional synthesis rate of mixed muscle proteins represents an average synthesis rate of several

cellular protein fractions including sarcoplasmic, mitochondrial and myofibrillar proteins. However, it is assumed that myofibrillar proteins such as myosin, actin, troponin, tropomyosin, titin and nebulin constitute approximately 70-80% by weight of skeletal muscle protein, and myosin alone comprises ~30% of muscle protein content (Balagopal *et al.*, 1997a). Thus, the synthesis rates of mixed muscle protein should reflect that of myofibrillar proteins. It has been reported that the stimulation of myofibrillar protein FSR during an amino acid infusion (Bohe *et al.*, 2001), and the increase of myosin heavy chain synthesis after exercise (Hasten *et al.*, 2000) have been closely related in magnitude to the increase in mixed muscle protein synthesis. Balagopal *et al.* (1997b) and Hasten *et al.* (1998) have reported myofibrillar protein synthesis to be ~72-80% of mixed muscle protein synthesis. The higher acute post-exercise increases of mixed muscle protein FSR compared to myofibrillar protein synthetic rates could be accounted by the higher turnover rates of mitochondrial and sarcoplasmic muscle proteins (Rooyackers *et al.*, 1996; Balagopal *et al.*, 1997b). Therefore, measuring mixed muscle protein synthesis provides an appropriate estimate of myofibrillar synthesis, which would account for muscle fibre hypertrophy.

Mean fibre area increased after training compared to both pretraining and untrained values. Significant hypertrophy of type IIA and IIX fibres were observed, along with a non-significant increase in type I fibre area. These findings are consistent with previous studies that reported a more pronounced type II fibre subtype hypertrophy (Staron *et al.*, 1989; Hather *et al.*, 1991; Kraemer *et al.*, 1995). Preferential type II muscle fibre hypertrophy may be dependent on the specifics of different high resistance training programs including the duration of training (Green *et al.*, 1998). Training volume could have limited the amount of type I fibre hypertrophy. Resistance training of at least 12 weeks induced muscle

hypertrophy of all three fibres (Staron *et al.*, 1989; Staron *et al.*, 1991; McCall *et al.*, 1996; Green *et al.*, 1998) while others have reported only a gradual increases after 8 weeks of training (Staron *et al.*, 1994). Thus, a high training volume (duration and frequency of workouts) of heavy resistance exercises appears to be necessary to induce hypertrophy in all muscle fibres.

Trends toward a decrease in type IIX fibres and a parallel increase in type IIA fibres were observed after training. MHC and ATPase histochemical analyses confirmed this gradual change in fibre type composition. Our findings coincide with other studies that have reported the fibre type transition (Staron *et al.*, 1989; Staron *et al.*, 1991; Adams *et al.*, 1993; Staron *et al.*, 1994; Carroll *et al.*, 1998). A transformation towards the MHC IIA isoform may represent a positive strength adaptation for repeated bouts of resistance exercises. It has been reported that in the vastus lateralis of individuals who have engaged in long-term resistance training contain a greater proportion of type IIA fibres (Staron *et al.*, 1984; Jurimae *et al.*, 1997). In a cross-sectional investigation of controls, weight lifters and distance runners, the volume percent of mitochondria in IIX fibres of runners and lifters were similar, yet significantly greater than in the controls (Staron *et al.*, 1984). Intuitively, the muscles of weight lifters performing multiple high-intensity, near maximal contractions would predominantly depend on highly glycolytic type IIX fibres. However, an increase in oxidative capacity of type II fibres is possible with resistance training (Staron *et al.*, 1984; Staron *et al.*, 1989). As discussed by Staron (1997) the assumption that classifications based on metabolic properties correlate with mATPase characteristics are not justified. Changes in oxidative capacity could, therefore, take place without accompanying changes in MHC (Staron *et al.*, 1984; Staron, 1997). These findings support the hypothesis that an increased

oxidative capacity of type IIX fibres could possibly precede the fibre shift to the type IIA fibre and potentially contribute to an advantageous strength adaptation during multiple high intensity bouts of resistance exercise (Staron *et al.*, 1984). Furthermore, the cumulative effects of repetitive exercise bouts could result in an adaptation of type II fibres to become more fatigue resistant due to an overall increase oxidative capacity (Staron *et al.*, 1984). Green *et al.* (1998) reported that high resistance training induced modest hypertrophy without compromising the capillarization or oxidative potential regardless of fibre type. This implies that resistance training may facilitate an increased oxidative potential within the muscle fibre, which can accommodate a greater proportion of type IIA (oxidative-glycolytic) and potentially provide a conducive environment of type IIX to IIA fibre transition.

A cross-sectional study, involving groups of competitive bodybuilders, resistance trained, endurance trained and control subjects, revealed a positive and negative correlation between relative MHC IIa and MHC IIX content and strength indices, respectively (Jurimae *et al.*, 1997). These data suggests a shift from MHC IIX to MHC IIa may be necessary for strength development and type II fibre hypertrophy to occur. Staron *et al.* (1994) concluded that if sufficiently stressed, alterations in muscle fibre composition could occur after only a few workouts and contribute to strength development. Resistance training involves maximizing power output over an extended number of exercise bouts (sets and repetitions). Thus, an increased proportion of type IIA fibres can be considered a favourable strength adaptation, which involves the trade-off of having a reduced peak power production with greater fatigue resistance.

The lack of significant changes in fibre type composition and distribution can be attributed to the initial proportions of type IIA and IIX fibres. A relatively low percentage of

type IIX fibres and higher percentage of type IIA prior to training could limit the degree of transformation. Perhaps a higher training volume (i.e. extend the training period, increase training frequency and/or exercise workload) would have resulted in significant changes. By grouping type IIAX fibres as type IIX, we could have underestimated the number of type IIA fibres and overestimated type IIX fibres. This miscalculation may have resulted in the low correlation coefficient between the post-training type IIA fibres and MHC IIA content. Thus, limitations within our methods could have led to the observed limited degree of fibre shifting.

Strength gains. Significant increases in strength measured as 1 RM leg press and knee extension exercises were observed after four weeks of resistance training. Although neuromuscular activity was not measured in this study, it has been generally accepted that early strength gains are attributed to increased neural adaptations (Sale, 1988; Chilibeck *et al.*, 1998; Akima *et al.*, 1999). It has been reported that isometric and isokinetic peak torques increased after two weeks of unilateral leg resistance training with no changes in muscle fibre type or fibre area (Akima *et al.*, 1999). The results indicated that improved motor unit recruitment induced greater peak torques after training (Akima *et al.*, 1999). However, other results shown strength gains without changes in neuromuscular activity using a similar unilateral training protocol after four weeks of training (Evetovich *et al.*, 2001; Ebersole *et al.*, 2002). This discrepancy could be accounted for by the methods used to measure the neural factors during and/or after resistance training. A reported increase in neural activity was measured using MRI (Akima *et al.*, 1999), whereas traditional EMG methods did not detect a change (Evetovich *et al.*, 2001; Ebersole *et al.*, 2002). MR images may be more sensitive to identify early changes in neural activity due to resistance training, as they can

evaluate muscle function in not only superficial muscle, which can be detected by conventional techniques such as EMG, but also a deep muscle (Akima *et al.*, 1999).

Physiological changes within the muscle may also play a role in early strength gains. Alterations within MHC isoform expression have been detected after two and four weeks of resistance training in men and women, respectively, with gradual increases in fibre CSA (Staron *et al.*, 1994). The common fibre type shift from type IIX to type IIA in trained muscle may contribute to the strength gain observed during early phases of resistance training. The addition of new contractile protein may occur after a few heavy workouts. Perhaps an initial increase in myofibril (thick and thin filaments) density, which may translate into a greater force generating capacity prior to an increase in measurable fibre area, could also contribute to early strength gains (Phillips, 2000).

Isokinetic strength measurements were used to compare changes in the trained and untrained leg before and after training. At 0.52 rad/s, significant increases in strength occurred after training in the trained leg only. This was an anticipated result and indicates that greater strength improvements are made during the same muscle movement used in training (Higbie *et al.*, 1996). Prior to training, there was no difference in peak torque production between legs. Isokinetic leg strength increased by 14% in the trained leg and remained unchanged in the control leg. These findings are consistent with those of Housh *et al.* (1992), Higbie *et al.* (1996) and Akima *et al.* (1999). There was no evidence of a cross-training effect from our results. The cross-training or cross-education effect occurs when strength training of one limb induces a significant increase in voluntary strength in both trained limb and untrained contralateral limb (Weir *et al.*, 1997; Taniguchi, 1998; Evetovich *et al.*, 2001; Shima *et al.*, 2002). The mechanism of cross-training has been originally

suggested to be a diffusion of motor impulses to the contralateral limb and postural contraction of the untrained limb to maintain balance and proper position for the unilateral exercise (Hellebrandt, 1951). In addition to central neural factors, peripheral factors, such as muscle hypertrophy may also contribute to increases in strength gains of the untrained limb (Housh *et al.*, 1992). The absence of a cross-training effect may reflect the relatively short training period and therefore the small increase in isokinetic strength observed in the trained limb. Also, subjects were intentionally limited in using their untrained limb for support while performing the training exercises thereby minimizing the possibility of postural contractions.

Strength was also markedly increased in the isotonic voluntary 1 RM compared to the isokinetic testing after training. An explanation for this is certainly due to the training specificity theory (Sale & MacDougall, 1981). Neural adaptations would provide greater motor unit activation of prime movers and the ability to better coordinate the activation of all relevant muscles to increase net force in the intended direction of movement (Sale, 1988). Since the movements were identical, a greater “training effect” would have taken place in the 1 RM testing for both training exercises.

2.5. Conclusion

In summary, 8 weeks of progressive unilateral leg training resulted in preferential type II fibre hypertrophy, unilateral muscle strength gains and elevated resting muscle protein synthesis rates in the T leg compared to the UT leg. In addition, the acute response to resistance exercise at the same relative workload was an attenuated increase in muscle protein FSR in the T versus the UT leg. These resistance training-induced adaptations appear

to be the result of an improved metabolic efficiency in order for muscle fibres to cope with subsequent exercise stress. However, the cellular and molecular mechanisms responsible for these adaptations are not well understood. Further investigations of muscle contraction-mediated molecular and cellular signaling in addition to nutritional and hormonal factors regulating muscle protein turnover are required.

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APPENDIX 1

**INFORMATION & CONSENT TO PARTICIPATE IN RESEARCH
FORM**

SUBJECT SCREENING QUESTIONNAIRE

DESCRIPTION OF MEDICAL PROCEDURES

EXERCISE METABOLISM RESEARCH GROUP
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

INFORMATION & CONSENT TO PARTICIPATE IN RESEARCH

***RESISTANCE TRAINING-INDUCED CHANGES IN HUMAN MUSCLE PROTEIN
SYNTHESIS AND FIBRE MORPHOLOGY***

You are being asked to participate in a research study being conducted by the investigators listed below. Prior to participating in this study you are asked to read this form which outlines the purpose and testing procedures and a separate form that describes the medical procedures (Description of Medical Procedures) used in this study. In addition, you must answer some questions regarding your health included in the attached forms (Subject Screening Questionnaire). Unless otherwise stated all testing and experimental procedures will be conducted in the Exercise Metabolism Research Laboratory, Rm. A103, Ivor Wynne Centre.

INVESTIGATORS:

Dr. Stuart Phillips
Dr. Mark Tarnopolsky
Paul Kim, B.Sc., B.Kin.
Sarah Wilkinson, B.Kin.

DEPARTMENT:

Kinesiology, IWC AB116
Medicine, MUMC 4U5
Kinesiology, IWC AB131A
Kinesiology, IWC A103

CONTACT:

x24465 or x27037
x24465 or x76367
x27390 or x27037
x27037

PURPOSE:

Resistance exercise (weightlifting) is known to increase muscle strength, muscle size and to cause a change in muscle fibre type. However, the mechanisms underlying these adaptations are not well understood in humans. To gain a better understanding of the mechanisms that affect human muscle protein synthesis post-exercise, an infusion of amino acids (protein), blood samples and muscle biopsies will be used combined with an 8 week one leg resistance training program.

DESCRIPTION OF TESTING PROCEDURES:

Prior to the commencement of the study you will be required to complete a routine medical screening (including a resting blood pressure measurement), a health questionnaire and two (2) baseline muscle biopsies taken from one leg and one (1) taken from the other leg (from the outside portion of your thigh) during the initial infusion. In addition you will have a familiarization session with the equipment and procedures involved in the study. After the familiarization session you will undergo a series of preliminary leg strength tests. The strength tests will all be performed on a Biodex (KinCom) apparatus, which will test your strength at a fixed speed, and will be applied to each leg individually. You will also be asked to abstain from any strenuous exercise for two days prior to undergoing the testing procedures. After the initial strength tests, you will have one leg randomly assigned as the leg to be trained. You will then participate in

an 8 week resistance training program, using only the leg that has been designated as that to be trained. This program will involve leg press (pushing with your leg) and knee extension (kicking with your leg) exercises. The amount of training will start with only two sets of each exercise, 8-10 repetitions at an intensity equivalent to 80% of what you can maximally perform (i.e., 80% of your 1 repetition maximum – RM – or single best lift). By the end of the 4th week of training you will be performing three sets of 8-10 repetitions and a fourth set to exhaustion and the intensity (i.e., % of your 1RM) will progressively increase, as you become stronger. Approximately 48 hours after the completion of the resistance training program, you will report to the Exercise Metabolism Research Lab in the Ivor Wynne Centre where two (2) muscle biopsies will be taken in the exercise-trained leg. Again, an isotope amino acid infusion will be done. You will report back to the lab 48 hours later to undergo an acute bout of exercise in both the trained and untrained leg and a final set of strength tests performed on the Biodex apparatus. Twelve hours later, the third set of biopsies will be done under infusion with two biopsies taken from each leg (4 in total). Hence, the total number of biopsies is nine (9) – each leg will have no more than 6 biopsies and no less than three. During the stable isotope infusions, a total of six (6) blood samples will be taken for a total of eighteen (18 or ~180mL or ~12 tablespoons) throughout the study.

POTENTIAL RISKS AND DISCOMFORTS

Please refer to the attached form entitled “Description of Medical Procedures” for a complete description of the medical procedures to be performed during the study and the potential risks associated with these procedures.

BENEFITS & REMUNERATION

In participating in this project you realize that there are no direct benefits to you. You will receive an honorarium, appropriate for the amount of time you have put into this project, of \$500 upon the completion of the study to compensate you for your time commitment (total of ~50h).

CONFIDENTIALITY

The blood and biopsy samples will be used for this research project only. All data collected during this study will remain confidential and stored in offices and on computers to which only the investigators have access. You should be aware that the results of this study will be made available to the scientific community, through publication in a scientific journal, although neither your name nor any reference to you will be used in compiling or publishing these results. Additionally, you will have access to your own data, as well as the group data when it becomes available and if you're interested.

PARTICIPATION & WITHDRAWAL

You can choose whether to participate in this study or not. You should be aware that your participation in this study will in no way affect your academic performance in any course offered within the Department of Kinesiology. You may exercise the option of removing your data from the study if you wish. You may also refuse to answer any questions posed

to you during the study and still remain as a subject in the study. The investigators reserve the right to withdraw you from the study if they believe that circumstances have arisen which warrant doing so.

RIGHTS OF RESEARCH PARTICIPANTS

You will receive a copy of this ethics form. You may withdraw your consent to participate in this study at any time, and you may also discontinue participation at any time without penalty. In signing this consent form or in participating in this study you are not waiving any legal claims or remedies. This study has been reviewed and received clearance from the Hamilton Health Sciences Corporation/McMaster University Research Ethics Board. If you have any further questions regarding your rights as a research participant contact:

REB Secretariat, CNH-111
McMaster University
1280 Main St. W.
Hamilton, ON
L8S 4L9

Tel: (905) 525-9140 x24765
Fax: (905) 540-8019
e-mail: grntoff@mcmaster.ca
<http://www.mcmaster.ca/ors/>

INFORMATION:

You will be able to contact Dr. Phillips at (905) 525-9140 (x24465 or x27037) and/or Dr. Tarnopolsky at (905) 521-2100 (X75226) or 24hr. pager (905) 521-2100 (X76443, Pager No. 2888) regarding any questions about the study.

I HAVE READ AND UNDERSTAND THE ABOVE EXPLANATION OF THE PURPOSE AND PROCEDURES OF THE PROJECT. I HAVE ALSO READ AND UNDERSTOOD THE ATTACHED FORM ENTITLED "DESCRIPTION OF MEDICAL PROCEDURES" AND COMPLETED THE ATTACHED FORM ENTITLED "SUBJECT SCREENING QUESTIONNAIRE" AND AGREE TO PARTICIPATE AS A SUBJECT. I HAVE ALSO RECEIVED A COPY OF THE INFORMATION AND CONSENT FORM. MY QUESTIONS HAVE BEEN ANSWERED TO MY SATISFACTION AND I AGREE TO PARTICIPATE IN THIS STUDY. I HAVE RECEIVED A SIGNED COPY.

SIGNATURE

DATE

PRINTED NAME OF WITNESS

WITNESS

DATE

PRINTED NAME OF WITNESS

INVESTIGATOR

In my judgment the participant in voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent and participate in this research study.

SIGNATURE OF INVESTIGATOR

DATE

EXERCISE METABOLISM RESEARCH GROUP
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

SUBJECT SCREENING QUESTIONNAIRE

Your responses to this questionnaire are confidential and you are asked to complete it for your own health and safety. If you answer "YES" to any of the following questions, please give additional details in the space provided and discuss the matter with one of the investigators. You may refuse to answer any of the following questions.

Name: _____ Date: _____

1. Have you ever been told that you have a heart problem?
YES NO
2. Have you ever been told that you have a breathing problem such as asthma?
YES NO
3. Have you ever been told that you sometimes experience seizures?
YES NO
4. Have you ever had any major joint instability or ongoing chronic pain such as in the knee or back?
YES NO
5. Have you ever been told that you have kidney problems?
YES NO
6. Have you had any allergies to any medications (including 'over-the-counter' medicines)?
YES NO
7. Have you had any allergies to food or environmental factors?
YES NO

8. Have you had any stomach problems such as ulcers?
- YES NO
9. When you experience a cut do you take a long time to stop bleeding?
- YES NO
10. When you receive a blow to a muscle do you develop bruises easily?
- YES NO
11. Are you currently taking any medication (including aspirin) or have you taken any medication in the last two days?
- YES NO
12. Is there any medical condition with which you have been diagnosed and are under the care of a physician (e.g. diabetes, high blood pressure)?
- YES NO
13. Have you previously participated in a study with Doctors Stuart Phillips, Mark Tarnopolsky, or Martin Gibala that involved having muscle biopsies taken?
- YES NO

EXERCISE METABOLISM RESEARCH GROUP
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

DESCRIPTION OF MEDICAL PROCEDURES

The study in which you are invited to participate involves three procedures, which require medical involvement: muscle biopsy sampling, stable isotope-labeled amino acid infusion, and venous blood sampling. Prior to any involvement, you are asked to read this form, which outlines the potential medical risks inherent to these procedures. In addition, you must also complete the "Subject Screening Questionnaire" which is designed to identify any medical reason, which might preclude your participation as a subject.

NOTE: If you have participated in another research study conducted by Dr. Stuart Phillips, Dr. Martin Cibala, or Dr. Mark Tarnopolsky that has involved muscle biopsies then you will not be allowed to participate in this study.

Muscle Biopsy Procedure

This procedure involves the removal of a small piece of muscle tissue using a sterile hollow needle. A medical doctor will clean an area over your quadriceps muscle (vastus lateralis) and inject a small amount of local anesthetic ("freezing") into and under the skin. He will then make a small incision (~4 mm) in the skin in order to create an opening through which to put the biopsy needle into your thigh. There is a small amount of bleeding from the incision, but this is minimal. The incision will be covered with sterile gauze and surgical tape. At those times during the experiment when a biopsy is required, the bandage will be removed and the medical doctor will insert the needle into your thigh through the incision. He will then quickly cut off a very small piece of muscle (~50-100 mg; about the size of the eraser on the end of a pencil) and remove the needle from your leg. During the time that the sample is being taken (~5 sec), you may feel the sensation of deep pressure in your thigh and on some occasions this is moderately painful. However, the discomfort very quickly passes and you are quite capable of performing exercise and any of daily activities.

Following the exercise bout, the biopsy sites will be closed with sterile bandage strips or a suture (stitch), and wrapped with a tensor bandage. You should refrain from excessive muscle use for the remainder of the day. Once the anesthetic wears off, your leg may feel tight and often there is the sensation of a deep bruise or "Charlie Horse". You should not take any aspirin-based medicine for 24 hours following the experiment as this can promote bleeding in the muscle. However, other analgesics (pain killers) such as Tylenol are acceptable alternatives. It is also beneficial to use a periodic application of an ice pack in the ensuing day, which will help to reduce any swelling and residual soreness.

The following day your leg may feel uncomfortable going down stairs. The tightness in the muscle usually disappears within 1-2 days, and subjects routinely begin exercising normally within 2-3 days. In order to allow the incisions to heal properly and minimize any risk of infection, you should avoid prolonged submersion in water for 2-3 days. Daily showers are acceptable, but baths, swimming, saunas, etc. should be avoided for at least the first 4 days following the biopsy procedure.

Potential Risks. The biopsy technique is routinely used in physiological research, and complications are rare provided that proper precautions are taken. However, there is a risk of internal bleeding at the site of the biopsy, which can result in bruising and temporary discoloration of the skin. On occasion a small lump may form under the site of the incision, but this normally disappears within 2-3 months. As with any incision there is also a slight risk of infection, however this risk is virtually eliminated through proper cleansing of the area and daily changing of wound coverings. If the incision does not heal within a few days or you are in any way concerned about inflammation or infection, please contact us immediately. In very rare occasions there can be damage to a superficial sensory nerve, which will result in temporary numbness in the area. There is also an extremely remote chance (1 in 1,000,000) that you will be allergic to the local anesthetic.

In past experience with healthy young subjects, 1 in 4,200 have experienced a local skin infection; 1 in 1,000 have experienced a small lump at the site of the biopsy (in all cases this disappeared within ~1 wk using massage); 1 in 1,750 have experienced a temporary loss of sensation in the skin at the site of incision (an area of numbness about the size of a quarter which lasted up to 3 months), and 1 in 100 have experienced mild bruising around the site of incision which lasted for ~4-5 days. While there is also a theoretical risk of damage to a small motor nerve branch of the medial vastus lateralis, this has never been seen in over 8,500 biopsies performed at McMaster University in the IWC. Hence, the risk of damaging a small motor nerve branch is impossible to estimate.

Catheterization and Venous Blood Sampling

A physician or a medically trained and certified member of the laboratory group will insert a small plastic catheter into a forearm vein. The catheter will be inserted with the assistance of a small needle, which is subsequently removed. The discomfort of this procedure is transient and is very similar to having an injection by a needle, or when donating blood. Once the needle is removed there should be no sensation from the catheter. During the course of the experiment, blood will be drawn periodically from the catheter. In any this experiment the total blood taken is less than 100 ml, which is approximately 1/5 of the blood removed during a donation to a blood bank. It is not enough of a blood loss to affect your physical performance in any way. After each blood sample has been taken, the catheter is "flushed" with a sterile saline solution in order to prevent blood from clotting in the catheter. This is a salt solution that is very similar in composition to your own blood and it will not affect you. Following removal of the

catheter, pressure will be placed on the site in order to minimize bleeding and facilitate healing.

Potential Risks. The insertion of catheters for blood sampling is a common medical practice and involves few risks if proper precautions are taken. The catheters are inserted under completely sterile conditions; however there is a theoretical risk of infection. There is a chance of internal bleeding if adequate pressure is not maintained upon removal of the catheter. This may cause some minor discomfort and could result in bruising/skin discoloration, which could last up to a few weeks. In very rare occasions, trauma to the vessel wall could result in the formation of a small blood clot, which could travel through the bloodstream and become lodged in a smaller vessel. However, we have never experienced such a complication after several thousand catheter placements.

Stable Isotope-labeled Amino Acid Infusion

You will receive, through a small catheter placed in your arm, an infusion (slow measured amount) of an amino acid (a small component of protein) solution. The amino acid will be dissolved in saline (a salt solution similar to your blood). The amino acid will be labeled with a stable isotope of carbon, hydrogen, or nitrogen. An isotope is slightly heavier form of these elements. Since the isotope is stable (i.e., non-radioactive) it poses no health risk to you due to radioactive exposure. Also, a certain fraction of all of the carbon, hydrogen, and nitrogen within your body is already in the same form as that of the stable isotope. Hence, the infusion of the stable isotope-labeled amino acid will simply result in a slight increase in the amount of stable isotope within your body; we refer to this as "enriching" the amount of stable isotope within your body. This enrichment will not remain high, however, and will be back to pre-infusion levels within 24 hours. All of the infused solutions are prepared under sterile conditions and are filtered through a very selective filter prior to entering your body. All solutions that enter your body do not contain, except for the amino acid, anything that will affect your health.

Potential Risks. Despite all precautions there is a theoretical risk that you could have a rapid drop in blood pressure due to some small bacterial contamination of the infusate. The risks of this drop in blood pressure are impossible to estimate. There is no calculable risk of the receiving the stable isotope-labeled amino acid.

APPENDIX 2

SUBJECTS' PHYSICAL CHARACTERISTICS

Subjects' Descriptive Characteristics

Subject	Age (years)	Weight (kg)	Height (m)	BMI (kg/m ²)	Dominant Leg	Training Leg
S1	29	73.5	1.65	27.00	Right	Left
S2	28	87.7	1.98	22.37	Right	Right
S3	30	98.6	1.94	26.20	Left	Left
S4	19	64.5	1.73	21.55	Right	Right
S6	25	69.1	1.75	22.56	Left	Right
S7	20	71.4	1.68	25.30	Right	Left
S8	19	62.3	1.82	18.81	Right	Right
S9	28	65.8	1.74	21.73	Left	Right
Mean	24.8	74.1	1.79	23.2		
SD	4.7	12.6	0.12	2.8		

Age, yr	24.8 ± 4.7
Weight, kg	74.1 ± 12.6
Height, m	1.79 ± 0.12
BMI, kg/m²	23.2 ± 2.8

APPENDIX 3

ISOKINETIC STRENGTH MEASUREMENTS AND ANOVA TABLE

1RM STRENGTH MEASUREMENTS AND ANOVA TABLE

Isokinetic Strength Measurements

Concentric 0.52 rad/s

Subject	Pre-UT (Nm)	Post-UT (Nm)	Pre-T (Nm)	Post-T (Nm)
1	184.7	198.8	220.1	307.0
2	322.1	299.5	300.6	302.2
3	254.2	207.5	250.4	209.2
4	211.1	180.8	184.6	187.5
5	155.8	152.9	141.8	183.0
6	200.2	217.2	197.9	248.5
7	223.5	225.0	209.0	225.0
8	156.0	138.9	146.1	176.6
Mean	213.5	202.6	206.3	229.9
SD	55.0	49.5	52.6	51.8
SE	19.5	17.5	18.6	18.3

Summary of all Effects

1-TIME (Pre vs. Post)

2-CONDITION (T vs. UT)

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	321.9453125	7	869.7803345	0.370145559	0.562138379
2	1	813.0527954	7	721.4978027	1.126895785	0.323684692
12	1	2371.832813	7	110.0078125	21.56103897	0.002360169

Tukey HSD test

Probabilities for Post Hoc Tests

INTERACTION: 1 x 2

	Pre-UT 213.5 kg	Post-UT 202.6 kg	Pre-T 206.3 kg	Post-T 229.9 kg
Pre-UT		0.24945426	0.557964683	0.063101172
Post-UT	0.24945426		0.889115393	0.005399108
Pre-TR	0.557964683	0.889115393		0.011791825
Post-TR	0.063101172	0.005399108	0.011791825	

1RM Strength Measurements

Knee Extension (kg)

Subject	Week 0	Week 3	Week 5	Week 7	Week 9
S1	37.5	40.0	60.0	67.5	72.5
S2	55.0	55.0	60.0	60.0	80.0
S3	37.5	45.0	70.0	82.5	85.0
S4	37.5	37.5	35.0	50.0	60.0
S6	25.0	42.5	60.0	70.0	80.0
S7	50.0	60.0	70.0	75.0	85.0
S8	45.0	55.0	65.0	70.0	70.0
S9	35.0	40.0	50.0	65.0	65.0
Mean	40.3	46.9	58.8	67.5	74.7
SD	9.4	3.5	11.6	9.7	9.3
SE	3.3	3.0	4.1	3.4	3.3

Knee Extension

Summary of all Effects

1-TIME (Week)

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	4	1610.390625	28	44.4977684	36.19036865	1.09854E-10

Tukey HSD test

Probabilities for Post Hoc Tests

MAIN EFFECT: TIME

	Week 0	Week 3	Week 5	Week 7	Week 9
	40.3 kg	46.9 kg	58.8 kg	67.5 kg	74.7 kg
Week 0		0.307396829	0.000182688	0.000133514	0.000133514
Week 3	0.307396829		0.010974467	0.00014019	0.000133514
Week 5	0.000182688	0.010974467		0.093174934	0.000569999
Week 7	0.000133514	0.00014019	0.093174934		0.226249039
Week 9	0.000133514	0.000133514	0.000569999	0.226249039	

Leg Press (kg)

Subject	Week 0	Week 3	Week 5	Week 7	Week 9
S1	145.0	145.0	180.0	195.0	220.0
S2	187.5	210.0	235.0	251.0	270.0
S3	130.0	130.0	160.0	170.0	200.0
S4	112.5	100.0	130.0	140.0	170.0
S6	80.0	115.0	145.0	155.0	200.0
S7	170.0	185.0	230.0	240.0	290.0
S8	170.0	195.0	220.0	230.0	260.0
S9	75.0	100.0	130.0	160.0	200.0
Mean	133.8	147.5	178.8	192.6	226.3
SD	42.2	43.8	44.3	42.8	42.1
SE	14.9	15.5	15.7	15.1	14.9

Summary of all Effects

1-TIME (Week)

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	4	10812.15039	28	113.0785751	95.61625671	6.63541E-16

Tukey HSD test

Probabilities for Post Hoc Tests

MAIN EFFECT: TIME

	Week 0	Week 3	Week 5	Week 7	Week 9
	133.8 kg	147.5 kg	178.8 kg	192.6 kg	226.3 kg
Week 0		0.100554168	0.000133514	0.000133514	0.000133514
Week 3	0.100554168		0.0001508	0.000133514	0.000133514
Week 5	0.000133514	0.0001508		0.095852733	0.000133514
Week 7	0.000133514	0.000133514	0.095852733		0.000137806
Week 9	0.000133514	0.000133514	0.000133514	0.000137806	

APPENDIX 4

Histochemistry Raw Data and ANOVA Table

Muscle Fibre Type Analysis Raw Data

Type I - Mean Fibre Area (μm^2)

	UT	Pre	T
1	4220	4501	4620
2	4765	3798	4479
3	4192	3856	5431
4	3268	4298	3188
5	3840	3852	3425
6	4156	5248	7219
Mean	4073	4259	4727
SD	495	562	1472
SE	202	229	601

Summary of all Effects

1-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	680590.75	10	629741.25	1.080746651	0.37589407

Type IIA - Mean Fibre Area (μm^2)

	UT	Pre	T
1	5480	5520	7648
2	7219	5618	7580
3	5222	5605	8970
4	4026	4178	4045
5	4691	5296	6605
6	5934	4732	6462
Mean	5429	5158	6885
SD	1097	584	1658
SE	448	238	677

Summary of all Effects

1-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	5176030.5	10	691165.125	7.488847733	0.010285802

Tukey HSD test - Probabilities for Post Hoc Tests

MAIN EFFECT: CONDITION

	UT	Pre	T
	5429	5158	6885
UT		0.842090726	0.031050444
Pre	0.842090726		0.012440622
T	0.031050444	0.012440622	

Type IIX – Mean Fibre Area (μm^2)

	UT	Pre	T
1	4328	4218	5251
2	5194	4663	7026
3	4851	4706	7659
4	3272	3394	3744
5	4416	4482	5905
6	5604	3133	5493
Mean	4611	4099	5846
SD	812	675	1384
SE	331	276	565

Summary of all Effects

1-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	4840101.5	10	501096.5625	9.65901947	0.004616634

Tukey HSD test

Probabilities for Post Hoc Tests

MAIN EFFECT: CONDITION

	UT	PRE	T
	4611	4099	5846
UT		0.452085972	0.031617463
PRE	0.452085972		0.004342437
T	0.031617463	0.004342437	

Type I - Percent Fibre Area (%)

	UT	Pre	T
1	31.1	24.1	22.1
2	30.5	20.4	35.9
3	35.5	49.9	40.0
4	39.1	48.1	47.5
5	21.7	28.5	35.6
6	26.6	35.5	18.0
Mean	30.7	34.4	33.2
SD	6.2	12.4	11.1
SE	2.5	5.1	4.5

Summary of all Effects

1-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	20.88663725	10	53.56399918	0.389938504	0.686959207

Type IIA - Percent Fibre Area (%)

	UT	Pre	T
1	40.4	40.4	45.6
2	49.8	44.5	35.5
3	26.0	23.8	43.2
4	56.3	35.0	47.8
5	46.1	40.0	34.1
6	66.4	41.2	59.3
Mean	47.5	37.5	44.3
SD	13.8	7.4	9.2
SE	5.6	3.0	3.7

Summary of all Effects

1-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	156.6838837	10	67.44855499	2.323013306	0.148388728

Type IIX - Percent Fibre Area (%)

	UT	Pre	T
1	28.4	39.1	32.3
2	19.7	35.1	28.6
3	38.5	26.3	16.8
4	4.6	16.3	4.7
5	32.2	31.5	30.3
6	7.0	23.3	22.8
Mean	21.7	28.7	22.6
SD	13.8	8.2	10.4
SE	5.6	3.3	4.3

Summary of all Effects

1-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	86.23500061	10	51.45033264	1.676082492	0.235635951

Type I - Percent Fibre Distribution (%)

	UT	Pre	T
1	33.9	25.3	28.6
2	54.9	25.7	47.8
3	39.7	56.4	51.4
4	43.7	46.0	52.7
5	24.4	33.7	40.0
6	30.3	23.7	15.7
Mean	37.8	35.1	39.4
SD	10.8	13.3	14.6
SE	4.4	5.4	6.0

Summary of all Effects

1-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	27.52383954	10	86.71588898	0.317403078	0.735109627

Type IIA - Percent Fibre Distribution (%)

	UT	Fre	T
1	32.3	30.7	35.3
2	14.6	38.2	27.8
3	22.9	18.8	33.3
4	51.3	34.2	42.6
5	41.7	33.7	30.3
6	63.2	48.9	57.9
Mean	37.7	34.1	37.9
SD	18.0	9.8	11.0
SE	7.4	4.0	4.5

Summary of all Effects

1-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	27.19388962	10	66.72988892	0.407521874	0.675862908

Type IIX - Percent Fibre Distribution (%)

	UT	Pre	T
1	33.9	44.0	36.1
2	30.5	36.2	24.3
3	37.4	24.8	15.3
4	5.1	19.8	4.6
5	33.9	32.6	29.7
6	6.6	27.4	26.4
Mean	24.5	30.8	22.8
SD	14.7	3.7	11.2
SE	6.0	3.5	4.6

Summary of all Effects

1-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	107.2866669	10	59.20999908	1.811968684	0.213052586

APPENDIX 5

Myosin Heavy Chain Content Raw Data and ANOVA Table

Myosin Heavy Chain Content Raw Data

UT			Pre					Post			
	I	Ila	Iix		I	Ila	Iix		I	Ila	Iix
S2-L	30.1	40.8	29.1	S2-R	23.9	39.0	37.1	S2-R	23.6	49.8	26.6
S3-R	33.5	50.3	16.2	S3-L	26.0	36.8	37.2	S3-L	35.3	39.5	25.2
S4-L	32.6	35.6	31.8	S4-R	45.1	31.9	23.0	S4-R	43.1	36.5	20.4
S6-L	39.0	53.6	7.4	S6-R	54.8	38.1	7.1	S6-R	45.6	47.4	7.0
S7-R	18.5	53.5	28.0	S7-L	33.0	35.0	32.0	S7-L	28.1	38.4	33.5
S8-L	18.5	65.7	15.8	S8-R	27.5	37.0	35.5	S8-R	31.4	41.5	27.1
MEAN	28.7	49.9	21.4	MEAN	35.1	36.3	28.7	MEAN	34.5	42.2	23.3
SD	8.4	10.6	9.6	SD	12.3	2.5	11.8	SD	8.6	5.3	9.0
SE	3.4	4.3	3.9	SE	5.0	1.0	4.8	SE	3.5	2.2	3.7

MHC Isoform Distribution (%)

MHC I - (%)

	UT	Pre	T
1	30.1	23.9	23.6
2	33.5	26.0	35.3
3	32.6	45.1	43.1
4	39.0	54.8	45.6
5	18.5	33.0	28.1
6	18.5	27.5	31.4
Mean	28.7	35.1	34.5
SD	8.4	12.3	8.6
SE	3.4	5.0	3.5

Summary of all Effects

1-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	74.44055176	10	33.91655731	2.194814682	0.162088409

MHC IIa - (%)

	UT	PRE	T
1	40.8	39.0	49.8
2	50.3	36.8	39.5
3	35.6	31.9	36.5
4	53.6	38.1	47.4
5	53.5	35.0	38.4
6	65.7	37.0	41.5
Mean	49.9	36.3	42.2
SD	10.6	2.5	5.3
SE	4.3	1.0	2.2

Summary of all Effects

1-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	279.831665	10	41.34366608	6.768428802	0.013843873

Tukey HSD test

Probabilities for Post Hoc Tests

MAIN EFFECT: CONDITION

	UT	Pre	T
	49.9	36.3	42.2
UT		0.011122108	0.143277526
Pre	0.011122108		0.296178639
T	0.143277526	0.296178639	

MHC IIx - (%)

	UT	Pre	T
1	29.1	37.1	26.6
2	16.2	37.2	25.2
3	31.8	23.0	20.4
4	7.4	7.1	7.0
5	28.0	32.0	33.5
6	15.8	35.5	27.1
Mean	21.4	28.7	23.3
SD	9.6	11.8	9.0
SE	3.9	4.8	3.7

Summary of all Effects

1-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	85.10055542	10	39.50522232	2.154159546	0.166746572

APPENDIX 6

Regression Analysis and Statistics Table

Regression Analysis -Percent Fibre Type Area vs. MHC content

SUMMARY OUTPUT	
MHC I vs % TYPE I AREA	
<i>Regression Statistics</i>	
Multiple R	0.83710438
R Square	0.700743743
Adjusted R Square	0.682040227
Standard Error	5.505106307
Observations	18

SUMMARY OUTPUT	
OVERALL UT	
<i>Regression Statistics</i>	
Multiple R	0.950173204
R Square	0.902829118
Adjusted R Square	0.896755938
Standard Error	5.017972718
Observations	18

SUMMARY OUTPUT	
MHC IIa vs % TYPE IIA AREA	
<i>Regression Statistics</i>	
Multiple R	0.790321532
R Square	0.624608124
Adjusted R Square	0.601146132
Standard Error	6.833537346
Observations	18

SUMMARY OUTPUT	
OVERALL PRE	
<i>Regression Statistics</i>	
Multiple R	0.808747979
R Square	0.654073293
Adjusted R Square	0.632452874
Standard Error	5.831270308
Observations	18

SUMMARY OUTPUT	
MHC IIx vs % TYPE IIX AREA	
<i>Regression Statistics</i>	
Multiple R	0.865070439
R Square	0.748346865
Adjusted R Square	0.732618544
Standard Error	5.795239031
Observations	18

SUMMARY OUTPUT	
OVERALL POST	
<i>Regression Statistics</i>	
Multiple R	0.866049157
R Square	0.750041142
Adjusted R Square	0.734418713
Standard Error	6.840991343
Observations	18

SUMMARY OUTPUT	
OVERALL MHC vs. FIBRE AREA	
<i>Regression Statistics</i>	
Multiple R	0.892918487
R Square	0.797303424
Adjusted R Square	0.793405413
Standard Error	5.894663788
Observations	54

APPENDIX 7

Mixed Muscle Fractional Synthesis Rate Raw Data and ANOVA Table

Mixed Muscle Fractional Synthetic Rate

Subject	UT-Rest	UT-Ex	Subject	T-Rest	T-EX
1	0.038	0.150	1	0.056	0.104
2	0.030	0.075	2	0.067	0.071
3	0.023	0.108	3	0.045	0.080
4	0.065	0.132	4	0.039	0.074
6	0.047	0.059	6	0.075	0.046
7	0.020	0.088	7	0.073	0.079
8	0.058	0.095	8	0.076	0.099
9	0.049	0.056	9	0.061	0.041
Mean	0.041	0.095	Mean	0.062	0.074
SD	0.016	0.034	SD	0.014	0.022
SE	0.006	0.012	SE	0.005	0.008

Summary of all Effects

1-TRAINING (UT vs. T)

2-CONDITION (REST vs. EXERCISE)

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	1.53125E-06	7	0.000414746	0.003692023	0.95324713
2	1	0.008944531	7	0.000948603	9.429164886	0.018049875
12	1	0.003423781	7	7.34241E-05	46.63020706	0.000246714

Tukey HSD test

Probabilities for Post Hoc Tests

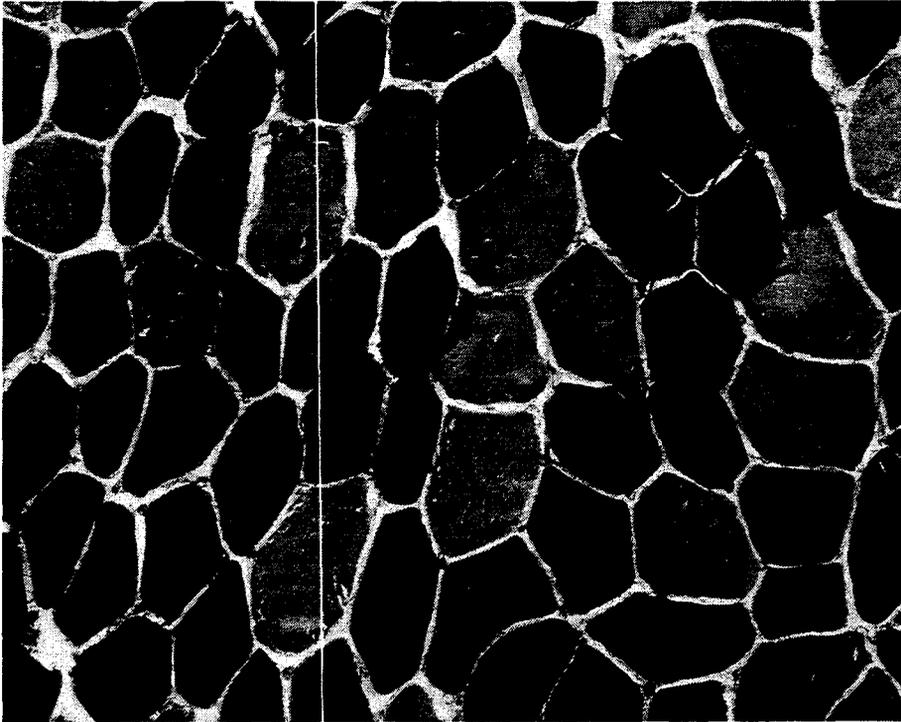
INTERACTION: 1 x 2

	UT-REST	UT-EXERCISE	T-REST	T-EXERCISE
	.041	.095	.062	.074
UT-REST		0.00026536	0.009043097	0.000690103
UT-EXERCISE	0.00026536		0.000617445	0.00722909
TR-REST	0.009043097	0.000617445		0.077360868
TR-EXERCISE	0.000690103	0.00722909	0.077360868	

APPENDIX 8

Histochemical Images

Subject 2 - Pre-Training - 200x



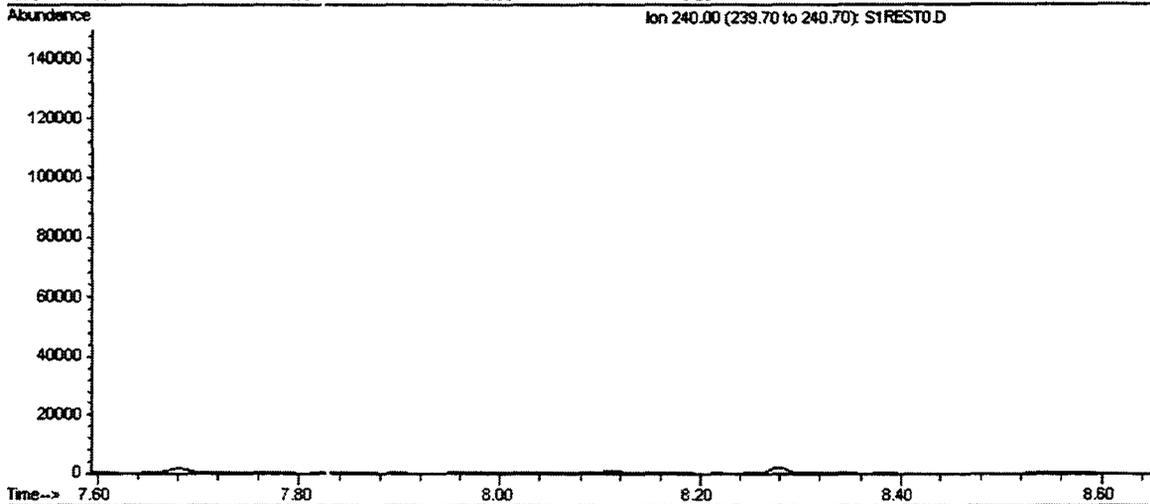
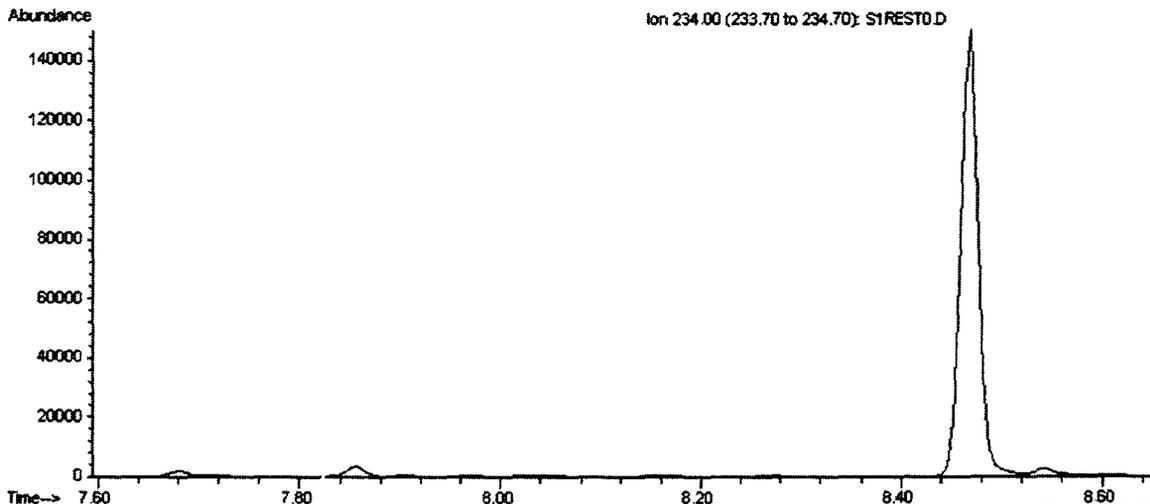
Subject 2 - Post-Training - 200x



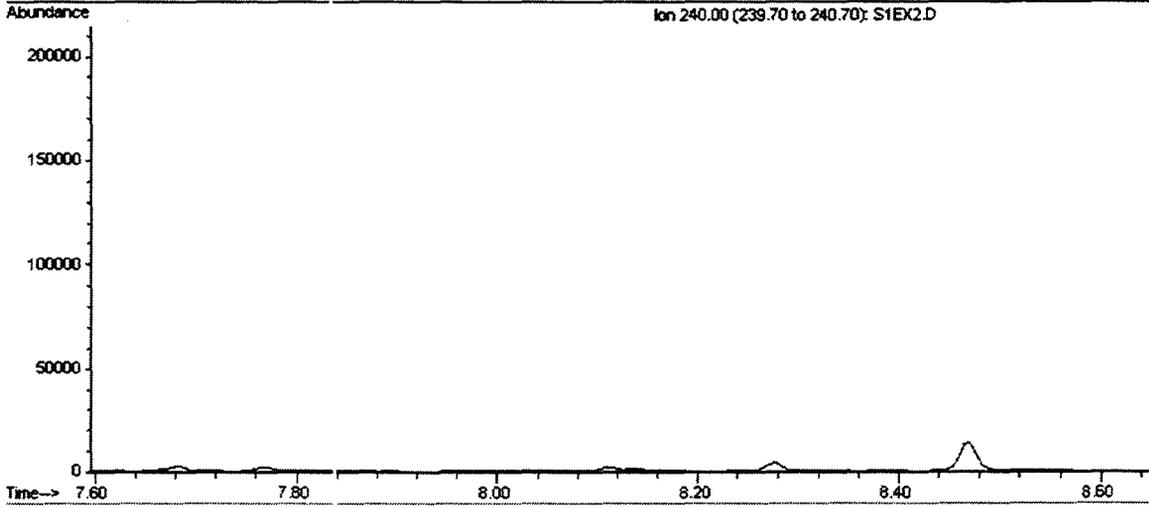
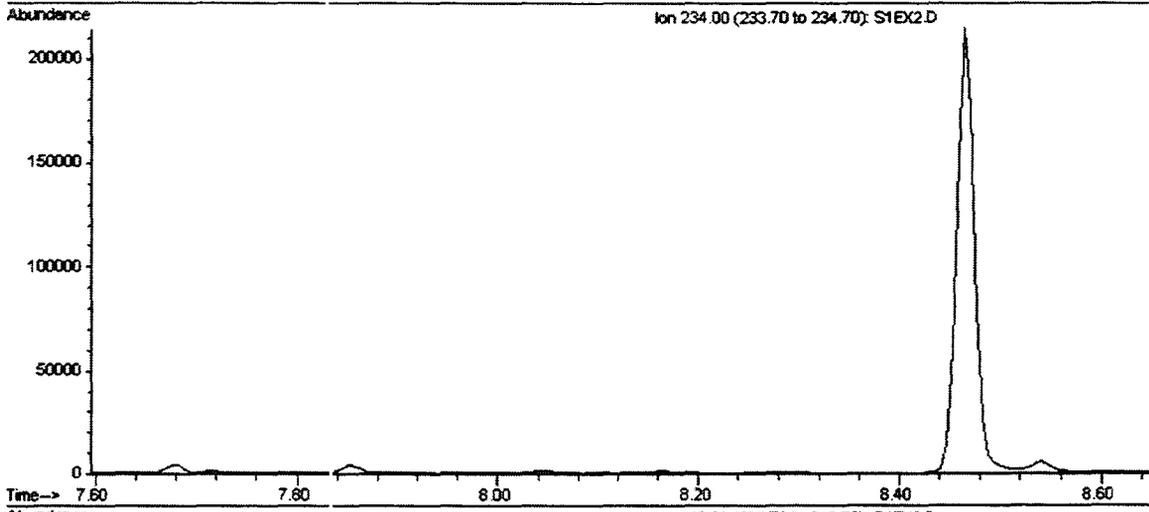
APPENDIX 9

Total Ion Chromatograms

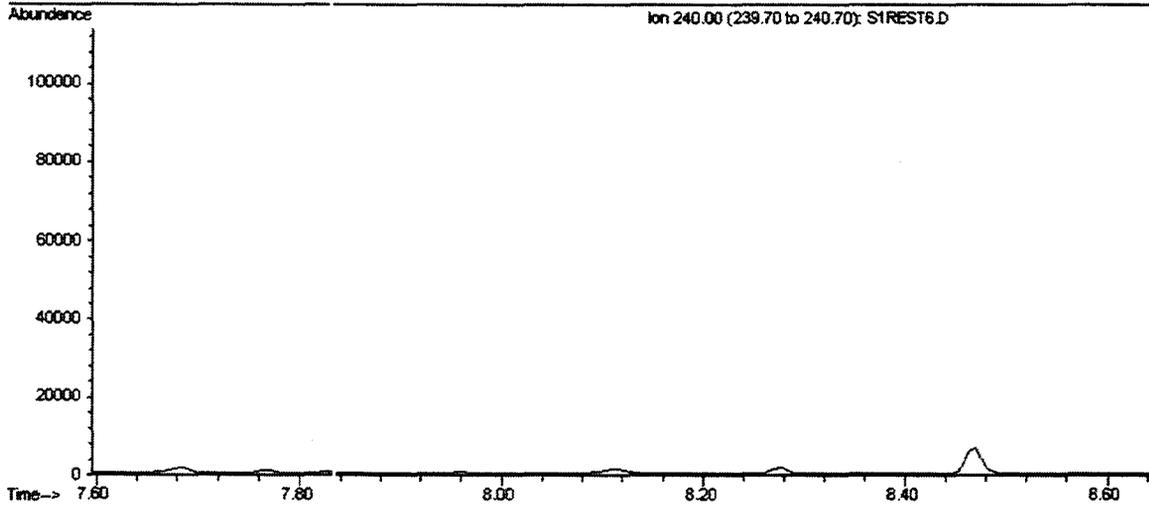
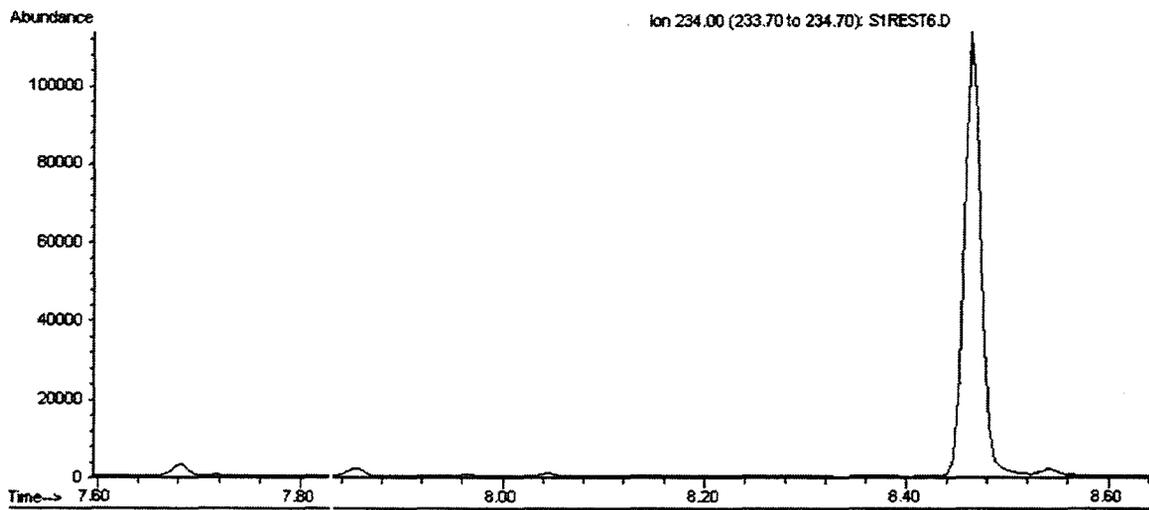
Plasma Enrichment S1 Rest – 0min



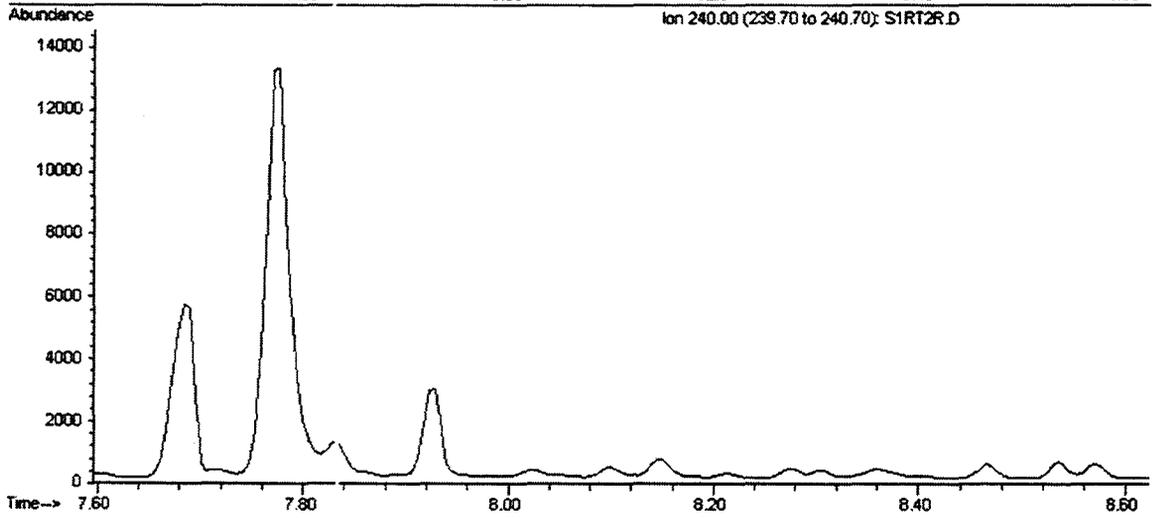
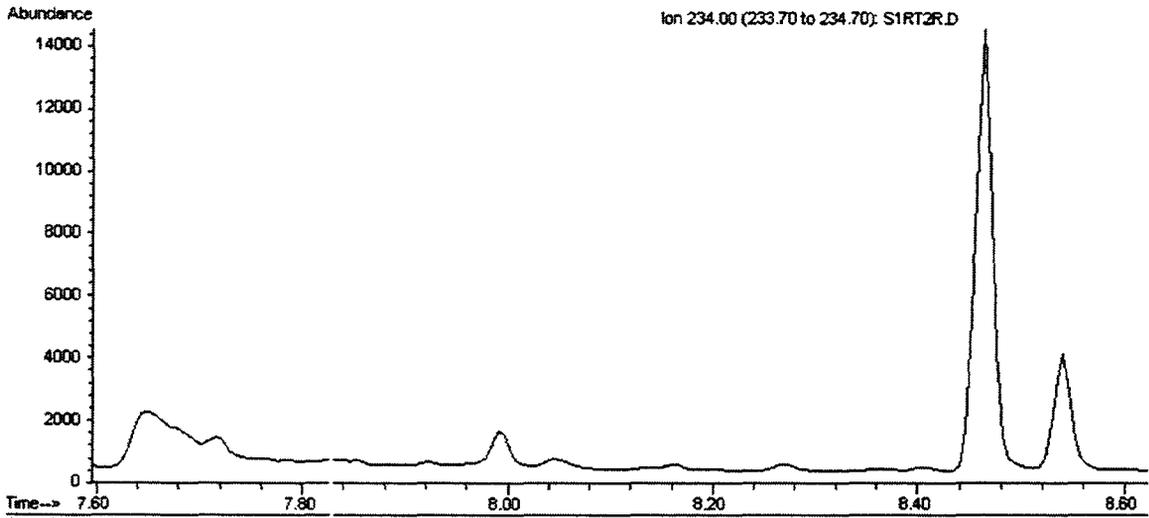
Plasma Enrichment S1 Rest – 120min



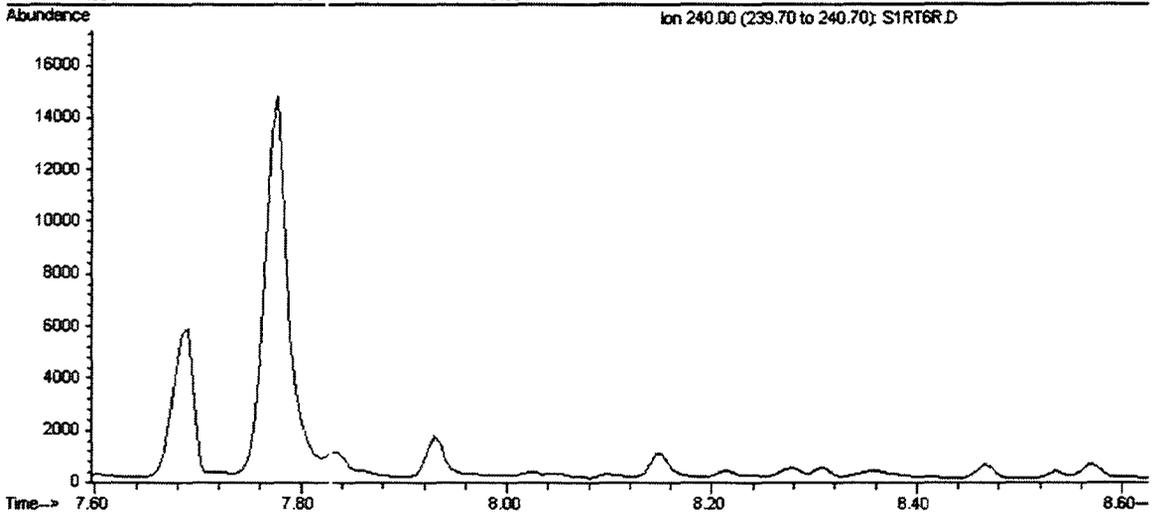
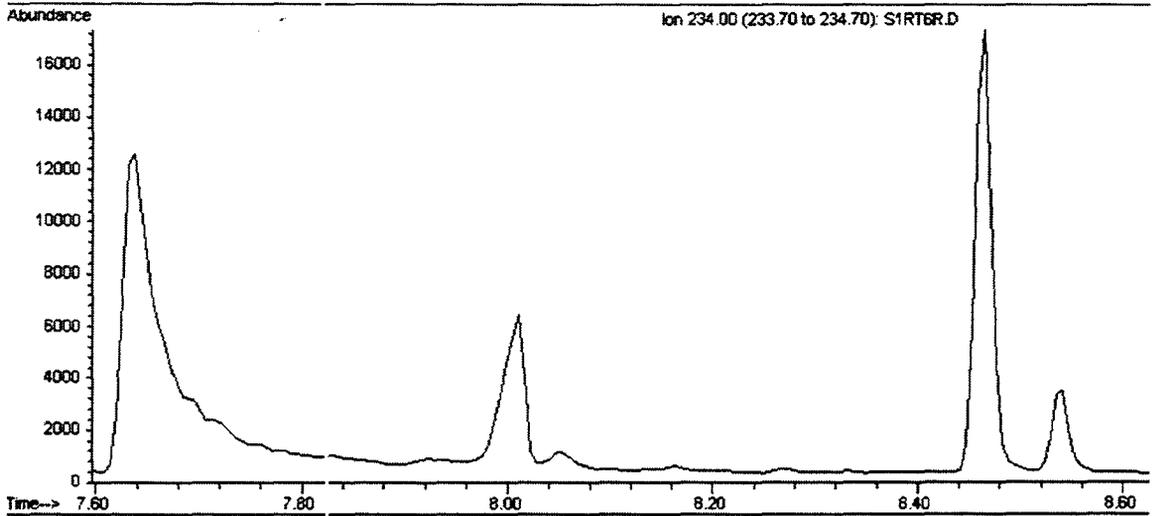
Plasma Enrichment S1 Rest – 360min



Intramuscular Enrichment S1-Rest-120min-UT



Intramuscular Enrichment S1-Rest-360min-UT



APPENDIX 10

MUSCLE FIBRE ANALYSIS/HISTOCHEMISTRY PROTOCOL

MUSCLE FIBRE TYPE ANALYSIS

Reference: Dubowitz, V. Muscle Biopsy: A practical approach 2ed. London: Bailliere Tindall, 1985.

Adapted by: Snow, R.J. School of Health Sciences, Deakin University, Australia

PART A: CUTTING MUSCLE

1. Store OCT mounted muscle at -80°C .
2. Prior to cutting muscle, place mounted muscle in cryostat for at least 15 to reach -20°C .
3. Trim the OCT covered portion of the sample at $30\mu\text{m}/\text{cut}$. Once muscle sample is exposed, reduce thickness of cut to $10\mu\text{m}$.
4. Cut 2-3 samples per slide.
5. Cover slides with paper towel and allow slides to dry overnight at 4°C .
6. Once dried, wrap slides in aluminum foil and store at -80°C until further analysis.

PART B: PREPARATION OF SOLUTIONS

Alkaline Stock Solution, pH 9.4

	Reagent	Manufacturer	Qty
1	Glycine	BioShop Biotechnology Grade - GLN 001	2.8163g
2	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	BDH 10070/EM Science 10070-34	3.00g
3	NaCl	BioShop Reagent Grade - SOD 002	2.1938g
4	NaOH	BDH Analytical Reagent ACS 816	1.3500g
5	MilliQ H_2O		500mL

1. Dissolve reagents in MilliQ H_2O and bring to volume.
2. Calibrate pH meter prior to adjusting pH to 9.4 with conc. HCl/5M KOH.
3. Store stock solution in fridge (4°C).

Acid Preincubation Stock Solution, pH 4.6

	Reagent	Manufacturer	Qty
1	Potassium Acetate	EM PX 1330-1	2.45g
2	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	BDH 10070/ EM Science 10070-34	1.30g
3	MilliQ H_2O		500mL

1. Dissolve reagents in MilliQ H_2O and bring to volume.
2. Calibrate pH meter prior to adjusting pH to 4.6 with glacial acetic acid.
3. Store stock solution in fridge (4°C).

5M NaOH (MW: 40.00g/mol) – Dissolve 20.00 g in 100 mL

5M KOH (MW: 56.11g/mol) – Dissolve 28.055 g in 100 mL

Alkaline Preincubation Solution

1. Remove alkaline stock solution from fridge and allow stock solution to reach room temperature.
2. Adjust pH of an appropriate volume (50mL) of alkaline stock solution to 10.50 using 5M NaOH (This should be done with continuous mixing and a pH meter sensitive to 0.001 pH units).

Acid Preincubation Solution

1. Remove acid stock solution from fridge and allow stock solution to get to reach room temperature.
2. Adjust pH of an appropriate volume (50mL) of acid stock solution to 4.30, 4.54, 4.60 with glacial acetic acid (This should be done with continuous mixing and a pH meter sensitive to 0.001 pH units).

ATP Preincubation Solution, pH 9.4 (PREPARE FRESH DAILY)

1. Add 170 mg of ATP (SIGMA A2383) to 100mL volumetric flask and bring up to volume using ALKALINE STOCK SOLUTION.
2. Adjust pH to 9.4.
3. Keep in fridge (4°C) until ready for use.

1% Calcium Chloride Stock Solution

1. Dissolve 10 g of $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ in 1000mL volumetric flask using MilliQ H_2O and bring up to volume.
2. Store at room temperature.

2% Cobalt Chloride

1. Dissolve 5 g of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ in 250mL volumetric flask using MilliQ H_2O and bring up to volume.
2. Cover in aluminum foil and store at room temperature.

1% Ammonium Sulfide (PREPARE FRESH DAILY)

1. Add 5 mL of 20% ammonium sulfide solution to 100mL volumetric flask.
2. Bring to volume.
3. Store in fume hood until ready for use.

PART C: FIBRE TYPE STAINING PROCEDURE

1. Incubate the sections in acid preincubation solutions adjusted to a pH of 4.30, 4.54 and 4.60; and alkaline preincubation solution adjusted to pH of 10.50 at the following time periods:

pH	Incubation time (min)
10.50	25
4.54	7.5
4.60	6.5
4.30	5.0

2. Transfer slides into plastic staining trough.
3. Rinse slides in distilled water **3 times**.
4. **Incubate slides in ATP incubation solution for 45 minutes at 37°C.** This should be done in a temperature-controlled shaker.
5. **Rinse slides in distilled water 2 times.**
6. **Incubate slides in 1% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Calcium Chloride) for 3 minutes** at room temperature.
7. **Rinse slides in distilled water 5 times.**
8. **Incubate slides in 2% $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (Cobalt Chloride) for 3 minutes** at room temperature.
9. **Rinse slides with distilled water 5 times.**
10. **Incubate slides in 1% ammonium sulphide for 1 minute** at room temperature.
11. **Rinse slides in distilled water 5 times.**
12. **Dehydrate tissue for 2 minutes** in each alcohol concentrations (70, 80, 90, 95 and 100% ethanol).
13. **Clear sections with xylene. Do this twice in clean xylene @ 2 minutes.**
14. **Blot off** excess xylene using Kimwipes. **Mount** the coverslips on slides using Permout (Fisher SP15-100). Allow Permout to dry (~1h). Store slides in the dark when not in use.

PART D: CAPTURING IMAGES / IMAGE ANALYSIS

1. Turn on camera and microscope and allow warming up for 5min.
2. Focus image at 4x magnification.
3. Refocus image at 20x magnification to calculate fibre area (μm^2).
4. Open SPOT Advanced software.
5. Click “Get Image” icon to capture image.
6. Click “Focus” icon to refocus image.
7. Save image as .jpg file.
8. Capture 3-4 images per sample.
9. When finished with microscope and camera:
 - a. Remove slide, lower platform, turn off camera, then microscope.
 - b. Replace lens and dust covers.
10. Use ImagePro Plus to determine fibre area (μm^2).

APPENDIX 11

PREPARATION OF BLOOD/PLASMA FOR ANALYSIS BY GCMS

PREPARATION OF MUSCLE FOR ANALYSIS BY GCMS

**PREPARATION OF MUSCLE FOR ANALYSIS BY GCMS: AMINO ACID
ISOLATION/PURIFICATION**

DERIVATIZATION OF PROTEIN HYDROLYSATE AND AMINO ACIDS

PREPARATION OF BLOOD/PLASMA FOR ANALYSIS BY GCMS:

NB: KEEP ALL SAMPLES AND REAGENTS ON ICE.

1. Allow appropriately labeled microcentrifuge (eppendorf) tubes to chill on ice (0-4°C).
2. Add 500µL of **ICE COLD** 0.6M Perchloric Acid (PCA) to microcentrifuge tube.
3. Add 200µL of plasma (100µL of whole blood) to PCA. Mix using vortex and cool on ice.
4. Centrifuge at 15,000rpm for 2 minutes.
5. Add 250 µL of **ICE COLD** 1.25M KHCO₃ (prepared **FRESH** daily) and let sit for 10 min. on ice **with lids open**.
6. Centrifuge at 15,000rcf (15,000g) for 2 min. at 0-4°C.
7. Remove supernatant and transfer into an appropriately labeled 13x100mm glass tube.
8. Dry samples in rotary evaporator (SpeedVac).

REAGENTS

1.25M KHCO₃

1.25g KHCO₃ in a 16x100mm disposable glass tube, add 10mL H₂O. Prepare **FRESH** daily

0.6M Perchloric Acid (PCA)

51.5mL 70% Perchloric Acid, bring up to 1000mL. Store 0-4°C for 2 months.

PREPARATION OF MUSCLE FOR ANALYSIS BY GCMS:

1. Cut a portion of frozen muscle sample in -20°C cryostat and transfer cut piece in appropriate labeled eppendorf tube with a punctured lid.
2. Freeze-dry samples overnight.
3. Record dry weight.
4. Manually powder dried muscle and remove any visible connective tissue.
5. Transfer powdered muscle into an appropriately labeled 13x100mm glass disposable tube. May store at -80°C until further analysis.
6. To extract intracellular amino acids:
KEEP ALL SAMPLES ON ICE.
 - a. Add 500µL of ICE COLD 0.6M PCA.
 - b. Gently shake or vortex to ensure powdered muscle has come into contact with PCA.
NOTE: Agitate carefully to minimize muscle adherence to the tube wall.
 - c. Let samples sit for 10min.
 - d. Centrifuge at 4500rpm for 2 minutes.
 - e. Using a P200 pipette, extract supernatant into an appropriately labeled eppendorf tube. Cap glass tube and save pellet for further analysis
 - f. Neutralize supernatant as blood/plasma samples:
 - i. Add 250 µL of ICE COLD 1.25M KHCO₃ (prepared FRESH daily) and let sit for 10min on ice **with lids open.**
 - ii. Let samples sit for 10min.
 - iii. Centrifuge at 15000g for 2min.
 - iv. Carefully decant supernatant into 13x100mm tube
 - v. Rotary evaporate samples for derivatization.

PREPARATION OF MUSCLE FOR ANALYSIS BY GCMS: AMINO ACID ISOLATION/PURIFICATION

Note: The muscle for this procedure can be either wet (must be maintained frozen) or dry.

REAGENTS

1. Dowex AG 100-200 mesh H⁺ form cation exchange resin
2. 4N NH₄OH
3. 1N HCl
4. 1N NaOH
5. pH paper (pH range 4-10)
6. Fisher Screening Column 11-387-50

COLUMN PREPARATION

1. Add ~2cm of resin (rinsed with DDI water) to commercial column – make sure the column has a **fritted filter** in the end prior to introducing resin.
2. Rinse the resin with **1 head volume of DDI water**.
3. Rinse the resin with **1 head volume of 2N NaOH**.
4. Rinse the column with at least **3 head volumes of DDI water**, or until **pH is neutral**. **Check for neutrality with pH paper**.
5. Rinse column with **2 head volumes of 2N HCl**.
6. **Neutralize** the column with at least **4-5 volumes of DDI water** - **CHECK** for neutrality with pH paper.

ABSORPTION/DESORPTION

1. Add sample (either 10% TCA intracellular extract, SSA precipitated blood, or **6N HCl muscle pellet hydrolysate**) to column.
Note: Add 0.5mL of hydrolysate, if dry weight of muscle is 1-2mg and 1mL of hydrolysate if <1.0mg.
2. Rinse the column with 7-8 head volumes of DDI water. **Wash vigorously to agitate beads and check for neutrality**.
3. Allow all fluid to escape for resin.
4. Desorb (release) the amino acids from the resin using 4N NH₄OH (~5mL).

5. Begin collecting the eluate from the columns immediately (i.e. have the collection tubes set up underneath the columns).
6. Evaporate sample using a rotary evaporator (approx. 12hrs). Be sure to use a BORIC ACID trap!
7. Store dry pellet – usually black/yellow in colour – at room temperature until ready for derivatization.

DERIVATIZATION OF PROTEIN HYDROLYSATE AND AMINO ACIDS

1. Add a mixture of 50 μ L MTBSTFA + 1% TBDMCS (Pierce P48925 or REGIS RT270142) + 50 μ L acetonitrile (HPLC grade) to dried samples.
Use Hamilton microliter glass syringe to transfer derivatizing agents.
2. Heat samples at 90°C for 1 hour.
3. Remove samples from heating block and allow cooling to room temperature.
4. Transfer derivative into appropriately labeled glass vials with inserts and cap. Use methanol to remove pen markings.
5. Place vials onto auto-sampler tray and run protocol.
6. Using Selected Ion Monitoring (SIM) mode, extract desired chromatographs.
7. Manually integrate peaks.
8. Record m/z ratios of selected ions using area under peak.

Notes on the operation of GC/MS

1. Rubber septa located under autoinjector may need to be replaced if target pulsed pressure value is not reached at the start of sample run.
2. Autotune before a series of samples or if GC/MS has been in use.
3. Clean syringe with methanol.
4. Change selected ions (m/z) in MS SIM/Scan Parameters.

APPENDIX 12

MYOSIN HEAVY CHAIN ELECTROPHORECTIC SEPARATION PROTOCOL

MYOSIN HEAVY CHAIN ELECTROPHORECTIC SEPARATION ANALYSIS

Lysing of Muscle

Reference: Staron RS, Hagerman FC, Hikida RS, Murray TF, Hostler DP, Crill MT, Ragg KE, Toma K. Fiber type composition of the vastus lateralis muscle of young men and women. *J Histochem Cytochem* 2000 May;48(5):623-9

The following is a procedure for obtaining tissue samples for MHC analysis:

1. Label 0.5 or 1 mL microcentrifuge tubes with permanent marker and place inside the cryostat to cool. Do not use tape to label the tubes.
2. Cut sections for MHC analysis. We usually cut sections serial to those we use for histochemistry. This is important because it assures us that we are sampling identical areas. We cut 4-6 sections (20 microns thick) for a **total of 80-120 microns**.
3. The number of sections cut for MHC analysis depends on the overall size and thickness. You may find it easier to just continue with what ever thickness you are using for histochemistry and just cut 8-12 sections for MHC analysis of the sample.

Our samples usually contain approx. 1000-1500 total fibers. More sections would obviously have to be cut for small biopsy specimens that have, for example, as few as 400 fibers.

It is better to have too much protein rather than too little. Therefore, a few "extra" sections are not bad. Try to place **approximately the same amount of tissue** in each tube. We can always dilute it later if that is necessary. Care must be taken to ensure that the **tissue does not thaw**. Use **toothpick** to place sections in the tube and **hold the centrifuge tube by the lid**.

Keep sectioned tissue (in tubes) in cryostat until you are ready to lyse.

4. Make sure the **tissue is at the bottom of the tube**.

Add approx. 250 μ L of lysing buffer [10% (wt/vol) glycerol, 5% (vol/vol) 2-mercaptoethanol, and 2.3% (wt/vol) SDS in 62.5 mM tris (hydroxymethyl) aminomethane HCl buffer (pH 6.8)]. **Lysing buffer should be kept cool on ice.**

Vortex and place in warm water bath for **10 min at 60°C**.

Make sure that the lids are closed tightly. We usually keep them upright in a small flask filled with water in the water bath. Make sure the tops of the tubes are not submerged.

5. Quick freeze immediately in liquid nitrogen and store at approx -80°C.

Preparation of Lysing Buffer

Reagents

- | | |
|---------------------------------|--|
| 1. TRIZMA HCl | Sigma T3253 (GMM: 157.6g/mol) |
| 2. Glycerol | BDH Inc. ACS 372 (Density: 1.2613g/mL) |
| 3. 2-mercaptoethanol | Sigma M6250 |
| 4. Sodium Dodecyl Sulfate (SDS) | EM Science (Merck KG9A) DX 2490-2 |

In 50mL volumetric flask:

Add: 0.4925g TRIZMA HCl (62.5mM)
4mL glycerol (10% (w/v))
2.5mL 2-mercaptoethanol (5% (v/v))
1.15g SDS (2.3% (w/v))

Bring to volume and **pH to 6.8**

Keep on ice.

Electrophoretic Separation

1. Small amounts of the extracts (3–5 μ l) were loaded on 4–8% gradient SDS-polyacrylamide gels with 4% stacking gels, run overnight (19–21 hr) at 120 V, and stained with Coomassie Blue.
2. MHC isoforms were identified according to their apparent molecular masses compared with those of marker proteins and migration patterns from single fiber analyses.
3. Relative MHC isoform content was subsequently determined using a laser densitometer.