THE EFFECT OF VELOCITY ON MUSCLE MORPHOLOGY FOLLOWING ECCENTRIC HIGH-RESISTANCE TRAINING IN YOUNG MALES

THE EFFECT OF VELOCITY ON MUSCLE MORPHOLOGY FOLLOWING ECCENTRIC HIGH-RESISTANCE TRAINING IN YOUNG MALES

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ABSTRACT

It is known that high-resistance training induces morphological changes in skeletal muscle. Following a resistance training program, increases in maximum torque generating capacity are observed due to both neural adaptations and hypertrophic gains within the trained muscle. Although it has been established that a muscle hypertrophies due to the addition of myofibrillar proteins through increased protein synthesis, the exact mechanism which stimulates the hypertrophic response is unknown.

Previous reports have shown that training in the absence of eccentric contractions generally produces less muscle growth and strength gains, as well as inflicting less damage to the muscle ultrastructure. Likewise, fast eccentric contractions have been shown to increase muscular strength to a greater extent than slow contractions. It has been hypothesized that fast eccentric contractions may maximize muscular damage, thus invoking a greater response of repair mechanisms, including satellite cell recruitment, which would allow an increased addition of contractile proteins to be added to the injured muscle, increasing muscle size and strength to a greater degree.

The effect of fast and slow eccentric training was investigated using a bilateral, within subject model. Twelve men trained one arm fast (3.66 rad/s) and one arm slow (0.52 rad/s) for 8 weeks on an isokinetic training apparatus. Type I muscle fibre size increased with training by an average of 9.3±12.0% ($P<0.05$, main effect for time). Type II muscle fibres increased more in the subjects' fast trained arm when compared to the slow trained arm according to ATPase histochemical analysis ($P<0.05$, time x condition interaction). Likewise, whole arm cross-sectional area showed that the fast trained arms had an average increase of 6.8±5.5 % whereas the slow arms only had an average

increase CSA of 5.1±5.7 % (P=0.065, time x condition interaction). Maximum torque generating capacity was also increased to a greater degree (P<0.05, time x condition interaction) in the fast trained arm with an average of 10.3±16.4 Nm, whereas the slow trained arm increased only 7.3±15.0 Nm, across testing speeds. A decrease in the percentage of type IIx fibres was seen in both arms after training according to both ATPase histochemical staining and MHC gel electrophoresis; however, the percentage of type IIa fibre area increased in the fast trained arms (8.4±8.6%) more significantly (P<0.05, time x condition interaction) than the slow trained arms (1.7±10.9%).

Seven males were trained in a similar manner to determine the extent of muscle damage which was evaluated by both Z-band streaming and force production decrements. After a single exercise bout of fast eccentric training in one arm and slow eccentric training in the other, it was determined that a 1.97±0.74 areas of moderate Z-band streaming per mm^2 of muscle in the fast exercised arm compared to 0.89±0.79 areas of moderate Z-band streaming per mm^2 of muscle in the slow trained arm (P<0.05). In conclusion, training using fast (3.66 rad/s) eccentric contractions causes a greater degree of muscle damage, hypertrophy, and strength gains than does training with slow (0.52 rad/s) eccentric contractions.

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LITERATURE REVIEW

1.1 Introduction

Chronic overload of skeletal muscle results in the muscle being able to generate greater maximal forces. The mechanisms underlying the gains in maximal force generation are twofold. The addition of contractile proteins, as well as neuromuscular adaptations, have been reported after resistance training, the combination of which results in greater strength (McCall et al., 1996; Widrick et al., 2002). Although strength gains seem to be specific to training type (Higbie et al., 1996; Hortobágyi et al, 1996), programs of eccentric muscle actions (generation of force during muscle lengthening) have been demonstrated to induce greater hypertrophic gains in muscle versus concentric contractions (generation of force during muscle shortening; Hather et al., 1991; Higbie et al., 1996; Hortobágyi et al, 1996a; Hortobágyi et al, 1996b). The mechanism of greater hypertrophy with eccentric-only training programs is unclear; however, it may be the result of increased muscle damage due to greater stress per active muscle fibre that occurs with eccentric contractions (Gibala et al., 1995). This greater damage would cause an increase in repair mechanisms, possibly including satellite cell activation, leading to the addition of a greater proportion of myofibrillar proteins and thus greater muscle hypertrophy.

It has been reported previously that resistance training implementing eccentric contractions of a higher velocity (3.14 rad/s) may bring about larger increases in muscle size (Farthing and Chilibeck, 2001) and strength (Paddon-Jones et al., 2001) compared to slower eccentric contractions (0.52 rad/s). The mechanism for the greater velocity- and training-dependent increase in muscle mass may also be the result of different degrees of

muscle damage. According to the force velocity relationship, faster eccentric contractions produce greater torque due to a positive-braking effect of the actin-myosin cross bridges.

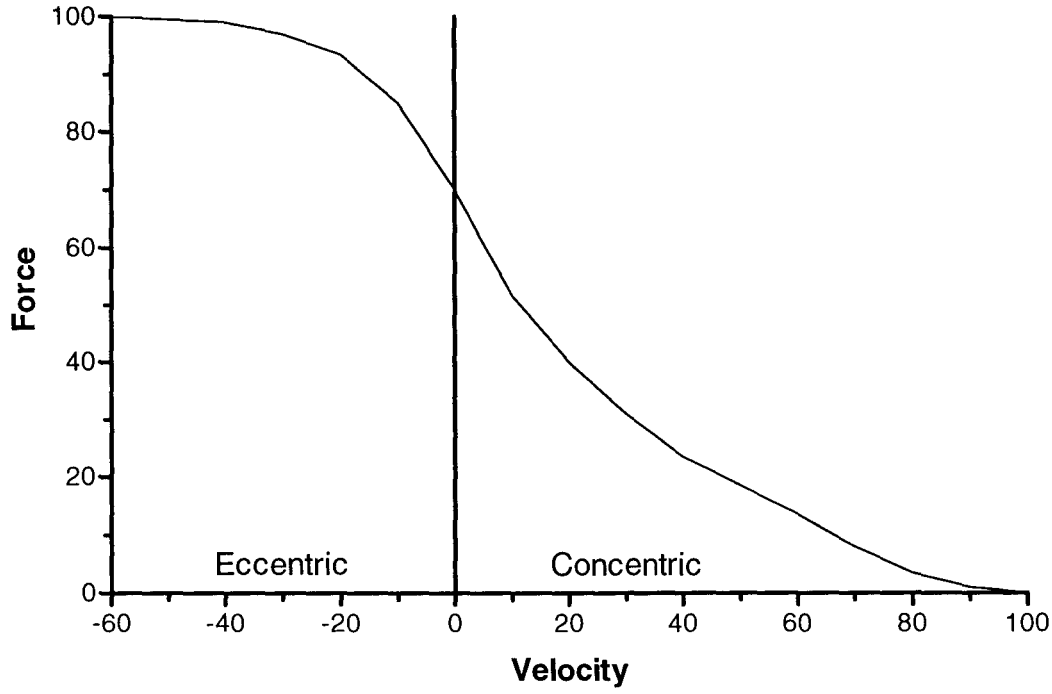

Figure 1. The force-velocity relationship.

Increasing the force placed on individual bound cross bridges may result in an augmentation of mechanical damage, further amplifying the local repair mechanisms resulting in greater hypertrophy.

1.2. Resistance Training

Resistance training is the most effective way to increase muscle mass and strength. Chronically overloading muscle causes both strength and hypertrophic gains in skeletal muscle. Specific techniques within a program of resistance training are less conclusive with respect to the optimal number of repetitions, sets and loads required to induce maximum strength or hypertrophic adaptations. It seems, however, that gains are

specific to training type. That is, training with a heavy load, low repetition regime induces high strength gains and muscle hypertrophy, whereas a lighter load, higher repetition protocol results in greater muscular endurance as opposed to strength, without the concomitant hypertrophy (Campos et al., 2002, Kraemer et al., 2002). The most advantageous length of rest periods both between sets and between training sessions is also somewhat inconclusive. Typically a three minute recovery period between sets is sufficient to resynthesize ~90% of key energy metabolites, such as PCr, that are depleted during a resistance training set (Fox et al., 1969). However, shorter rest periods may recruit additional muscle fibres, thus stressing a greater number of fibres. Number of training sessions per week should give adequate amounts of recovery time, but more advanced programs can be more frequent due to a decreased incidence of muscle damage with training (Kraemer et al., 2002).

1.2.1 Muscle Strength

Muscle strength can be improved as early as 2 days after the onset of resistance training (Chilibeck et al., 1998; Staron et al., 1994). An increase in muscle strength due to resistance training occurs whether measured as maximal isometric torque or as a dynamic voluntary one-repetition maximum, and is a result of both neural adaptations and muscle hypertrophy (Colliander and Tesch, 1990; Higbie et al., 1996; Hortobágyi et al., 1996a; Hortobágyi et al., 1996b; McCall et al., 1996). A hypertrophied muscle has a greater volume of contractile proteins, or myofibrils, allowing more actin and myosin heads to bind and thus generating higher forces. Although hypertrophy does contribute to strength gains, it is generally accepted that early increases in force generating capacity are produced as a result of neural adaptations. Motor unit synchronization is one neuromuscular

adaptation that is hypothesized to contribute to initial strength gains seen with resistance training. By increasing the synchronization of motor unit firing, muscles are able to produce greater force during rapid contractions, and may also function to coordinate multiple muscles in a synergistic fashion (Semmler, 2002). Other adaptations may also include increased neural activation, and a more efficient firing pattern, which would also contribute to a greater force production (Enoka and Fuglevand, 2001).

1.2.2. Muscle Hypertrophy

Scientists have attempted to elucidate whether the resistance training-induced increase in muscle size is due to either an increase in fibre size and/or an increase in fibre number, termed hyperplasia (Taylor and Wilkinson, 1993). However, to date there have been no verified accounts of hyperplasia in humans. An increase in muscle CSA is accounted for by adding new contractile proteins, namely actin and myosin, within an individual muscle fibre, therefore hypertrophying that fibre (McCall et al., 1996; Widrick et al., 2002). At least 6 weeks of continuous resistance training is required before significant increases in CSA of both whole muscle and individual muscle fibres can be detected using current methodology (Hortobágyi et al., 1996a; McCall et al., 1996; Staron et al., 1994). It is, however, logical to assume that small but potentially physiologically significant increases in fibre CSA (at least from a force generating perspective) do occur at earlier time points, but due to the variability of measurement techniques, are not recognized (Phillips, 2000).

1.2.2.1 Protein Balance

The addition of muscle protein can only occur if the muscle is in a state of net anabolism, which means muscle protein synthesis must be greater than muscle protein breakdown. An increase in protein synthesis occuring following resistance exercise is augmented further with the ingestion of dietary protein. Phillips at al. (1997) demonstrated an elevated mixed muscle protein fractional synthetic rate (FSR) of 112%, 65% and 34% above resting levels at 3 h, 24 h and 48 h respectively, after an acute bout of resistance exercise. Protein fractional breakdown rate (FBR) was also elevated 31% at 3 h and 18% at 24 h, but was not significantly different from rest at 48 h post exercise. Similar time courses for elevated protein synthesis have also been demonstrated by others (e.g., MacDougall et al., 1995).

Resistance training, combined with the ingestion of either essential amino acids (Tipton et al., 1999) or essential amino acids combined with carbohydrate at both 1 h and 3 h post exercise (Rasmussen et al., 2000), have been shown to increase FSR during the first few hours following a bout of resistance exercise, but not the rate of muscle protein breakdown. In a recent study, Tipton et al. (2001) demonstrated that essential amino acid and carbohydrate ingestion prior to a bout of resistance exercise may cause a greater net protein synthesis than the same supplementation immediately after resistance training. This may in part, be due to an increased delivery of amino acids to the exercised muscle.

1.2.2.2 Hormonal Control of Muscle Growth

Although net protein synthesis is required for muscle growth, other factors including various hormones and growth factors can influence the rate of protein synthesis and breakdown. Substances that have been shown to result in an increased muscle protein

FSR include testosterone (Ferrnando et al., 2002; Ferrnando et al., 2003), growth hormone (Russell-Jones et al., 1998), insulin (Biolo et al., 1999; Fryburg et al., 1995), and insulin-like growth factor-1 (IGF-1; Fryburg et al., 1995). Likewise, glucocorticoids, which have been shown to increase with inactivity (Ferrando et al., 1999), are able to increase FBR, resulting in a less positive or a negative net protein balance (Paddon-Jones et al., 2003).

1.2.3. Muscle Fibre Types

Myosin is the most abundant protein expressed in skeletal muscle and comprises ~25% of total protein within a striated muscle. Each myosin molecule is composed of two heavy chains (MHC) and two pairs of light chains (MLC) twisted together in a helical fashion. One end of each heavy chain is folded into a globular head that binds to specific sites on an actin to form the actin/myosin cross-bridge which propagates muscle contraction. The function of myosin is to form the backbone of the contraction apparatus, as well as serving as the functional motor of the contraction itself. The contraction is initiated through the ATPase action of myosin, which translates chemical energy into mechanical action, thus propagating the shortening of the sarcomeres to conclude a muscle contraction (Baldwin and Haddad, 2001).

There have been numerous MHC isoforms discovered that are expressed by muscle fibres, giving each muscle fibre different contractile and biochemical characteristics according to the distinct ATPase properties displayed by that MHC isoform. The distinct properties that are associated with a particular MHC ATPase translate into varied functional properties of each individual isoform. The general isoforms that have been shown to be expressed by electrophoretic analysis of MHC in human skeletal muscle are: slow type I, fast type IIa, fast type IIx or IIb (Baldwin and Haddad, 2001).

1.2.3.1 MHC Isoform Characteristics

The common terms used to describe type I and II fibres are slow and fast fibres respectively. It has been determined that the maximum shortening velocity (V_{max}) of individual muscle fibres is partially dependent upon the MHC isoform that is expressed within that fibre. Each MHC isoform has its own ATPase activity, which determines the rate of release of chemical energy from ATP. Therefore, the greater speed at which the enzyme can hydrolyze ATP, the faster a mechanical action can be propagated thus quickening the rate at which a muscle can contract. According to this principle, studies that have been completed on maximum shortening velocities of muscle fibres have shown that type I fibres are slower than the type II fibres. Fibres that contain predominantly type IIx MHC isoform have been shown to have the greatest V_{max} (Bottinelli et al., 1996; Larson and Moss, 1993; Harridge et al., 1996).

Similar to the maximum shortening velocity that a muscle fibre can attain, the MHC isoform a fibre expresses also has a potential effect on the maximum contractile force that a fibre is able to produce. It has been well established in the literature that type I fibres produce less force then type II fibres. However, it is less conclusive as to whether there is a significant difference in power generation between type IIa and IIx fibres (Bottinelli et al., 1996; Larson and Moss, 1993). Most, if not all, of the differences in maximal force generating capacity between fibres can be accounted for by differences in CSA. Typically, type II fibres are associated with a greater cross sectional area than their type I counterparts (Staron et al., 2000).

1.2.3.2 Muscle Fibre Type Transitions

Initially in an untrained, sedentary muscle there are a relatively high percentage of fibres expressing the IIx MHC isoform. This MHC isoform has been proposed to be the 'default gene' that is expressed in situations of disuse, inactivity, or reduced weight bearing (Baldwin and Haddad, 2001). Further support for is evidenced by the significantly higher proportion of type IIx fibres seen in studies involving limb immobilization (Hortobágyi, et al., 2000), the use of bed rest (Berg et al., 1997), and in spinal cord injured subjects (Talmadge et al., 2002; Andersen et al., 1996). After as little as 4 weeks of endurance training, however, the number of fibres that express the IIx isoform is decreased significantly, and in most subjects 'pure' type IIx fibres are totally eliminated (Klitgaard et al., 1990). A general summary of the training studies that have been performed indicate that the MHC isoform transitions tend to be in the IIx to IIa direction, possibly moving through the hybrid (single fibres expressing multiple isoforms) fibre isoforms. Twelve weeks of progressive resistance training has been shown to decrease the proportion of hybrid fibres and result in a far greater percentage of 'pure' fibres expressing only one MHC isoform (Williamson et al., 2000; Williamson et al., 2001). In spinal cord injured patients functional electrical stimulation (FES) has been shown to change fibre types from almost complete type IIx dominance to type IIa dominance (Andersen et al., 1996). It is rare to see fibre type transitions, in healthy humans that involve the transition from IIa to I (Baldwin and Haddad, 2001).

1.3. Eccentric Versus Concentric Contractions

Eccentric muscle contractions require lower activation of the nervous system to achieve the same torque as a concentric contraction. Electromyogram (EMG) recordings indicate that eccentric contractions recruit fewer motor units than concentric contractions to obtain a similar muscle force (Caruso et al., 2001; Grabiner et al., 2002). Likewise, eccentric contractions have greater work efficiency (work per unit of energy expended) and fatigue resistance than concentric contractions (Caruso et al., 2001). Grabiner et al. (2002) suggested that the central nervous system differentiates between maximal eccentric and concentric contractions, and that an a priori activation of motor units may occur supraspinally. A centrally controlled, predetermined contraction type may influence activation patterns and alter motor unit recruitment, and may be the cause of eccentric contractions having less antagonistic muscle involvement compared to concentric actions. Less antagonistic muscle involvement was seen in eccentric contractions as opposed to concentric contractions, particularly at higher contraction velocities; this was identified as an important factor in achieving a greater maximal voluntary contraction force with eccentric contractions (Kellis and Baltzopoulos, 1998).

1.3.1 Eccentric Strength

Muscle is able to produce more torque when forcibly lengthened than when shortened (Hather et al., 1991; Higbie et al., 1996; Hortobágyi et al., 1996a; Hortobágyi et al., 1996b; Hortobágyi et al., 2001). Greater torque generation with eccentric contractions is mainly accounted for by mechanical advantages at the myofibril level. When contracting eccentrically some cross-bridges are not cycled and instead are continually being pulled backward, which may disrupt the actomyosin bonds mechanically rather than

undergoing an ATP-dependent detachment (Enoka, 1996; Flitney and Hirst, 1978). Hence, such cross-bridges function to slow the lengthening of the muscle. Since the cross-bridges are being bent back, the myosin heads do not rotate forward, allowing the actin and myosin to remain bound and additional cross-bridges to be formed, producing more tension without needing to recruit additional motor units (Stauber, 1989).

The size principle, which suggests that small motor units have a lower recruitment threshold, and are thus recruited prior to larger motor units, is generally accepted for concentric contractions. However, evidence has been presented that high threshold motor units may be recruited preferentially during an eccentric contraction (Nardone et al., 1989). Similarly, recent data by Linnamo et al. (2002) also suggests that eccentric contractions may preferentially recruit larger, fast motor units when a muscle is preactivated. At lower force levels eccentric contractions had higher mean spike amplitudes indicating greater recruitment of fast motor units, which were then able to enhance force production, not by recruiting additional motor units, but instead by increasing the firing rate, as shown by the increasing mean spike frequency with increasing force (Linnamo et al., 2002). Other studies have shown similar results (McHugh et al., 2002), suggesting that a better ability to recruit fast motor units may also contribute to the superior ability to generate force during an eccentric contraction.

It is generally accepted that contraction type specific training is particular to increasing strength for that mode of training. It seems that eccentric training increases eccentric strength more than concentric training increases concentric strength (Higbie et al., 1996; Hortobágyi et al., 1996a; Hortobágyi et al., 1996b). Likewise, after eccentric specific training greater isometric forces are able to be produced than similar training using concentric contractions (Hortobágyi et al., 1996a). Stated more broadly, eccentric training

causes more generalized gains, across the force-velocity spectrum, in strength compared to concentric training.

Upon completion of 12 weeks of unilateral, maximal leg resistance training in a concentric (CON) or eccentric (ECC) group, ECC subjects increased EMG activity significantly more than those in CON (Hortobágyi et al., 1996b). Although ECC increased EMG activity during concentric testing equivalent to the increase of CON during eccentric testing, a difference was detected when the testing mode was specific to that training type (i.e. during eccentric testing after ECC, EMG was increased 7-fold versus concentric testing after CON). Similar increases in EMG activity have demonstrated more recently after similar eccentric training (Hortobágyi et al., 2001). These findings (Hortobágyi et al., 1996b; 2001) support the notion that eccentric training may produce more significant neuromuscular adaptations, which may in part be due to a greater capacity for learning with eccentric contractions, possibly because of the relative infrequency of their use in daily activities.

1.3.2 Eccentric Contraction-Induced Hypertrophy

Eccentric only resistance training elicits greater hypertrophic gains in skeletal muscle than that of concentric only (Hather et al., 1991; Higbie et al., 1996; Hortobágyi et al., 1996a; Hortobágyi et al., 1996b; Hortobágyi et al., 2001), although not all studies have illustrated this (Mayhew et al., 1995). Higbie et al. (1996) randomly assigned 16 women to a concentric group, 19 women to an eccentric group and 19 women to a control group and trained them accordingly for 10 consecutive weeks. Women trained in the eccentric group had a 6.6% increase in leg CSA determined from magnetic resonance images (MRI), compared to a 5% increase in the concentric group ($P<0.05$).

Greater hypertrophy seen with eccentric training most likely due to the greater stress, or higher forces placed on muscle fibres. Fewer motor units are recruited for an eccentric contraction of the same force magnitude as a concentric contraction (Caruso et al., 2001; Grabiner et al., 2002) resulting in a larger force per active muscle fibre, which results in greater damage (Gibala et al., 1995). It is possible that this stress 'overload', which creates damage in skeletal muscle, is a key signaling mechanism to increase muscle protein synthesis (Chen et al., 2002, Haddad and Adams, 2002). One study showed no correlation between protein synthesis and myofibrillar disruption induced by eccentric or concentric contractions (Gibala et al., 2000), however, there has been report of an increased concentration of muscle IGF-1 mRNA, as well as a decrease in IGF binding protein-4 (IGFBP-4) mRNA concentration 48 hours after a bout of eccentric exercise versus concentric exercise (Bamman et al., 2001). It is plausible that the reason for greater muscle hypertrophy after eccentric training is through greater mechanical stress per muscle fibre, thus signaling IGF-1 to increase downstream mechanisms that stimulate muscle hypertrophy.

1.3.3 Velocity of Eccentric Contractions

Maximal contractions at high velocities may elicit greater gains in both strength (Paddon-Jones et al., 2001) and muscle thickness (Farthing and Chilibeck, 2001) when compared to slower contractions. Preliminary research from Farthing and Chilibeck (2001) investigated the difference in muscle adaptation between isokinetic dynamometer training implementing fast (3.14 rad/s) and slow (0.52 rad/s) eccentric or concentric contractions. Untrained subjects (n = 24) were randomly assigned to a fast or slow training group in which they trained the elbow flexors of one arm eccentrically and the other arm

concentrically for 8 weeks. Fast (3.14 rad/s) eccentric training resulted in greater muscle thickness, using B-mode ultrasound as an assessment tool, than did either velocity trained in the concentric mode. Slow (0.52 rad/s) eccentric training resulted in greater muscle thickness over fast concentric, but not slow concentric training. The authors concluded that fast eccentric training may be the most beneficial training mode with regard to muscle hypertrophy, although they admitted that due to the between subject variability they may have been underpowered to detect a difference between slow and fast eccentric conditions with only six arms, and a mixed design, per condition (P. Chilibeck, personal communication).

Data from Paddon-Jones et al. (2001) demonstrated a larger increase in strength gains following training with fast (3.14 rad/s) versus slow (0.52 rad/s) eccentric contractions. Subjects were assigned to either a fast or slow training group in which they completed 24 eccentric unilateral elbow flexor contractions 3 times a week for 10 weeks. Isometric strength increases were apparent ($P<0.05$) in the fast group, but were not significant ($P>0.05$) in the slow group after 10 weeks of training. In contrast, concentric torque did not increase for either group at 0.52 rad/s, after 5 weeks of training, but both fast and slow groups did have a significant increase in torque at 3.14 rad/s. However, following the remaining 5 weeks of training, the fast group continued to increase concentric torque at 3.14 rad/s, whereas the slow group returned almost to baseline values by the end of the 10 week training period. Eccentric torque values were attenuated over the 10 week period for the fast group at both testing velocities, but were not significant for the slow group at any time point. The authors suggested that this gain in strength may be due to greater neural adaptation during the fast training, due to an initial inhibitory/protective neural mechanism seen in eccentric contractions (Stauber, 1989;

Hortobágyi et al., 1996b), which could be more prevalent during the faster contraction velocities.

Some rather 'unconventional' results from this study (Paddon-Jones et al., 2001) were reported for the muscle fibre type transitions that occurred during the 10 week training period. Although the slow group did not show any muscle fibre type transitions, the fast training group demonstrated an increase in the percentage of type IIx fibres while seeing an analogous decrease in the number of type I fibres. The authors suggested that this unusual observation may be a result of an adaptation to chronic selective recruitment of type IIx fibres used in the high velocity eccentric contractions (Paddon-Jones et al., 2001). More likely, however, is that the subjectivity associated with classifying fibres along with the fact that the number of fibres identified in the analysis was likely insufficient, resulted in a type 1 statistical error.

1.4. Exercise Induced Muscle Damage

It has been previously determined that strenuous eccentric contractions result in significant amounts of muscle damage. Data from one laboratory showed that both untrained (Gibala et al., 1995) and trained (Gibala et al., 2000) individuals experienced significant amounts of ultrastructural damage after eccentric, but not concentric contractions, when compared to baseline. Greater muscle damage during eccentric contractions can be attributed to higher stress per muscle fibre that occurs during an eccentric contraction, due to less motor unit recruitment (Grabiner et al., 2002). Many different measures have been used to estimate the amount of damage that occurs within a muscle after eccentric contractions. Force deficits, muscle enzymes within the blood, and ultrastructural damage to the muscle are the most common encountered in the literature.

1.4.1. Force Decrements

Prolonged strength loss is considered to be one of the most reliable indirect methods for determining the existence of muscle damage (Warren et al., 1999a). Unaccustomed eccentric contractions result in significant reductions in maximal force generating capacity, whether measured voluntarily or involuntarily (Michaut et al., 2002; Warren et al., 1999b). Sayers and Clarkson (2001) complied data from 192 subjects, who had completed a bout of damaging eccentric contractions using their elbow flexors, which resulted in an average of 57% decrease in maximum voluntary contractile force (MVC) immediately after the exercise. At 132 h (5.5 d) post exercise, 67% of the subjects' MVC strength had returned. While performance of multiple eccentric contractions results in prolonged fatigue, by 10 days post exercise strength is usually seen to return to pretraining values. However, when subsequent bouts of eccentric contractions were completed 7 days after the initial session, an additional force drop lasting up to 1 to 2 extra days is seen, rather than the 10 d force reduction after the first exercise session (Nosaka and Newton, 2002). In contrast, concentric contractions only cause an immediate decrease in force generating capacity, which is rapidly restored (Clarkson et al., 1992; Gibala et al., 1995).

A prolonged loss of force seen after eccentric muscle actions has been hypothesized to be caused by a disruption in the protein structures within the muscle or more likely the excitation-contraction (EC) coupling process (Warren et al., 1999), whereas immediate strength losses, such as those seen following concentric contractions, are attributed to metabolic or neural fatigue (Clarkson and Hubal, 2002).

1.4.1.1 Disruption of Force Generating Capabilities

After a bout of forceful eccentric contractions, there is disruption of sarcomeric proteins (Z-band streaming), as well as damage to the EC coupling system. Warren et al. (2001) hypothesized, based primarily on animal data, that 75% or more of the force deficit seen after eccentric resistance exercise is due to the disruption of the EC coupling system. Supporting evidence for their hypothesis is demonstrated in a mouse model, where caffeine was used to recover the lost tension within the muscle (Balnave and Allen, 1995). The addition of caffeine releases calcium from the sarcoplasmic reticulum (SR) and allows the muscle to contract, thereby indicating that it was not physical damage to the force generating structures that led to the decline in tension. Although it is apparent that EC coupling disruption affects the force generating capacity of skeletal muscle, the specific site of failure is uncertain, but is suggested to be between the plasmalemma and the SR calcium release channel (Warren et al., 2001).

1.4.2 Muscle Enzyme Release

The most common muscle enzyme measured in the blood to indicate the occurrence of muscle damage is creatine kinase (CK). The presence of CK in the blood is indicative of muscle injury because CK is normally only contained in the muscle cell, and must then be a result of a disruption in the muscle membrane (Clarkson et al., 1992). While CK occurrence in blood may be an indication of membrane leakage from damaged muscle, this does not necessarily reflect myofibrillar disruption, whereas the presence of MHC fragments or troponin I may correlate more closely with direct markers of muscle injury (McNeil and Khakee, 1992).

1.4.3 Myofibrillar Disruption

Although it seems evident that damage to the EC coupling system may contribute more significantly to the fall in maximal tension seen after eccentric exercise, damage to the muscle ultrastructure is also present, and possibly the only direct measurement of muscle damage. Ultrastructural damage to muscle fibres is most predominately seen as misalignment, or "streaming" of Z-bands (Beaton et al., 2002; Gibala et al., 1995; Gibala et al., 2000). Z-band streaming can be seen using electron (Gibala et al., 1995; Gibala et al., 2000) and light microscopy, although the latter has a high inter-site variability (Beaton et al., 2002). The magnitude of disruption to the Z-band has frequently been categorized as focal (1-3 adjacent sarcomeres), moderate (3-10 sarcomeres), or extensive (greater than 10 adjacent sarcomeres) (Gibala et al., 1995; Gibala et al., 2000).

The mechanism of Z-band streaming is somewhat controversial; however, one possibility is that during an eccentric contraction, the sarcomeres are stretched to a point that is beyond the length of the actin and myosin filaments' overlap (Russell et al., 1992; Best and Hunter, 2000). This stretch is speculated to involve the elastic filament titin, or the structural protein desmin (Proske and Morgan, 2001). By stretching beyond the actin-myosin overlap, titin which anchors the thick filament to the Z-discs may be severed under the mechanical stress. Likewise, it is also possible that desmin, which links adjacent sarcomeres to one another, may be the weak point and may be disrupted causing the Z-band streaming. Whatever the mechanism might be, the result is a misalignment of Z-bands, which can result in overstretched sarcomeres that may butt up against one another and become ineffective in generating force (Proske and Morgan, 2001).

Another result of stretching past the actin-myosin overlap is causing damage to the nearby sarcolemma, which in turn allows for an influx of extracellular calcium ions.

This unregulated influx of calcium into the cell results in the activation of calcium-dependent proteases, called calpains, which are able to cleave proteins such as the myofibrillar and cytoskeletal proteins (Beaton et al., 2002a; Tidball, 1995). In turn there may be further myofibrillar disruption that can also cause a distortion of I- and A-bands.

1.4.4 Muscle Soreness

Eccentric exercise is usually accompanied by delayed onset muscle soreness (DOMS), which typically begins approximately 6-8 hours post exercise, and peaks at about 48 hours (Proske and Morgan, 2001). DOMS is usually associated with muscle damage; however, recent research has indicated that it does not correlate well with direct markers of muscle damage, such as increased MHC concentration in the blood (MacIntyre et al., 2001; Nosaka et al., 2002). Correlation between DOMS and direct markers of muscle damage has not been studied specifically. DOMS has been suggested to be a direct result of the inflammation of an injured muscle, which is triggered by muscle damage. A muscle inflammatory marker interleukin-6 (IL-6) has recently been positively correlated with DOMS, supporting the theory of DOMS being related to muscle inflammation, not damage as indicated by the presence of MHC content in blood. The authors' hypothesis was that DOMS was only present with higher concentrations of IL-6 indicating inflammation, whereas increased incidence of MHC in the blood was the marker for mechanical muscle damage (MacIntyre et al., 2001).

1.5. Muscle Inflammatory Cell Response

One of the first reactions to muscle injury is the infiltration of macrophages, fibroblasts, and other neutrophils. The substance that attracts the inflammatory cells, in the case of muscle injury, is not known for certain. One known chemoattractant for inflammatory cells is platelet derived growth factor (PDGF), which is released into the extracellular space following muscle injury. This growth factor could account, at least in part, for the early inflammatory cell response following muscle damage (Tidball, 1995).

Regardless of the mechanism of stimulation for inflammatory cell infiltration, neutrophils are the first cells to respond to the muscle injury. It has been shown that neutrophils invade the injured muscle as early as 2 h and have peaked by 6 h post injury in response to eccentric muscle contractions (MacIntyre et al., 2001). The primary role of the neutrophil is to remove any debris that has been left in the muscle, in a phagocytotic fashion; however, other roles of the neutrophils have also been hypothesized. Other possible functions of neutrophils are to produce cytokines, such as interleukin (IL)-8[4] and tumor necrosis factor (TNF)α, and oxygen-derived free radicals such as such as superperoxide ($O\cdot^{-}_{2}$), hydrogen peroxide (H_2O_2), nitric oxide ($NO\cdot$), and hydroxyl radicals ($HO\cdot$). The purpose of these cytokines and free radicals is likely to provide a signal for monocyte invasion (Best and Hunter, 2000).

Research has shown that macrophages exist in two populations; $ED1^+$ and $ED2^+$. The $ED1^+$ macrophages exist in low concentrations in healthy muscle close to the surface of the muscle fibers in a quiescent state, but are in higher abundance in muscles that contain necrotic fibers (Tidball, 1995). The close proximity of $ED1^+$ macrophages to muscle cells suggests that they may be 'sensors' of the muscle injury. When these macrophages are activated, they not only function to phagocytose injured muscle, but also

to secrete growth factors such as transforming growth factor α (TGF-α), TGF-β, IL-1α, IL-1β, and PDGF, which are all chemoattractants for other inflammatory cells (Tidball, 1995). The other subpopulation of macrophages, $ED2^+$, do not perform phagocytosis in the injured muscle, but instead function as sources of growth factors and cytokines, such as insulin-like growth factor (IGF), IL-6 and PDGF, which may in turn function to stimulate and regulate satellite cell proliferation (Best and Hunter, 2000; Cantini et al., 1994).

1.5.1. Regeneration of Damaged Muscle

The muscle stem cell, termed the satellite cell, acts synergistically with protein synthesis, and subsequent protein accretion, in the regeneration of damaged muscle to ensure that there is a constant nuclear-to-myoplasmic volume ratio (Rosenblatt et al., 1994; White and Esser, 1989). If the basal lamina of an injured muscle fiber is still intact, satellite cells fuse to existing multinucleated myotubes, which then differentiate into mature muscle fibers with peripherally located nuclei (Campion, 1984; Hawke and Garry, 2001). Alternatively, if the basal lamina is not intact, differentiated satellite cells may be able to fuse to each other forming an entirely new myotube, as is seen in embryonic muscle (Schultz, 1989). Although muscle hyperplasia, particularly as a result of resistance training, has never been confirmed in human subjects, the fusion of satellite cells to form new myotubes is one hypothesized mechanism behind how hyperplasia could occur (Antonio and Gonyea, 1993; Taylor and Wilkinson, 1986).

Before being inserted as a muscle nucleus, the satellite cell must be activated from its quiescent state, proliferate, and differentiate into a myoblast. The satellite cell is likely to be activated by hepatocyte growth factor (HGF) (Bischoff, 1989; Sheehan et al., 2000; Tatsumi et al., 1998). Proliferation may then be due to the presence of fibroblast growth

factor (FGF) (Allen et al., 1984) and/or macrophage-derived growth factor (MDGF) (Chambers and McDermott, 1996). IGF-1 and testosterone which are known to maintain and increase muscle mass (Adams and Haddad, 1996; Ferrnando et al., 2003; Fryburg et al., 1995, Goldspink, 1999), by increasing protein synthesis, have also been implicated in the proliferation and differentiation of satellite cells (Chambers and McDermott, 1996).

Satellite cells have been deemed necessary for hypertrophy to occur in skeletal muscle. Rosenblatt and Parry (1994) overloaded the extensor digitorum longus (EDL) of mature rats by removing the synergist, tibialis anterior (TA) muscle. Ablation of the TA caused compensatory hypertrophy of the EDL; however, irradiating the EDL, thus incapacitating the satellite cells, completely eliminated hypertrophy. While synergist ablation is a rather 'severe' model to induce hypertrophy, the fact that hypertrophy was not seen when satellite cells were made dysfunctional highlights their importance in the hypertrophic process.

1.6. Rationale and Hypotheses

Strength gains as a result of resistance training are due to early neuromuscular adaptations and later hypertrophic gains (Colliander and Tesch, 1990; Higbie et al., 1996; Hortobágyi et al., 1996a; Hortobágyi et al., 1996b; McCall et al., 1996). While the exact contribution of each factor is not known, hypertrophy is due to the addition of newly synthesized muscle proteins (Phillips, 2000). It has been previously determined that eccentric contractions place a greater stress per active muscle fibre (Grabiner et al., 2002) and cause ultrastructural damage (Gibala et al., 1995), which may in fact be the mechanism for the greater hypertrophic gains seen with eccentrically biased training protocols (Hather et al., 1991; Higbie et al., 1996; Hortobágyi et al., 1996a; Hortobágyi et al., 1996b;

Hortobágyi et al., 2001). Greater damage is likely to cause an increase in muscle regenerative processes, possibly involving satellite cell involvement (Jacobs et al., 1995; Rosenblatt et al., 1994; Schultz, 1989; White and Esser, 1989).

Training with higher velocity eccentric contractions has been shown to result in increased muscle strength gains (Paddon-Jones et al., 2001) and possibly greater hypertrophy (Farthing and Chilibeck, 2001) in comparison to training with slower eccentric contractions. The mechanism of greater strength increases, and slow to fast fibre type shifts, may be due to the continual recruitment of fast motor units forcing them to adapt to the chronic training by increasing in size and number (Paddon-Jones et al., 2001). Increased type II fibre size would further contribute to an increase in muscle strength as well as an increase in the percent area and type II MHC content. Greater muscle hypertrophy (Farthing and Chilibeck, 2001) due to higher velocity eccentric training could possibly be due to a greater relative stress placed on individual muscle fibres, compared with slower velocity eccentric contractions, thus causing more myofibrillar damage. During eccentric contractions as the Z-bands are being stretched farther apart, the myosin heads function to slow down the lengthening process. By forcing the lengthening to occur more quickly, as would occur with more rapid eccentric contractions, myosin heads may not be able to release from their actin binding sites in time, causing even more ultrastructural damage than would occur with slow eccentric contractions.

The effect of high velocity contractions on muscle hypertrophy has been studied using B-mode ultrasound (Farthing and Chilibeck, 2001), but has not been examined at the muscle fibre level. High (3.14 rad/s) versus low (0.52 rad/s) velocity eccentric contractions have been reported to increase the percentage of IIx fibres in the bicep brachii muscle of untrained men (Paddon-Jones et al., 2001), contrary to the vast majority of

published reports which have demonstrated that MHC shifts from fast (IIx) to slower (IIa) MHC isoforms (Andersen et al., 1996; Demirel et al., 1999; Klitgaard et al., 1990). Further, the effect of high velocity contractions on muscle damage has not been considered in previous research. Therefore the purpose of this study was to examine the effect of fast (3.66 rad/s) and slow (0.35 rad/s) eccentric contraction velocities on muscular properties, including hypertrophy, strength, fibre type, myosin heavy chain expression, and myofibrillar damage. We chose a 10-fold difference in the eccentric velocities used during training, in an attempt to maximize differences between the two conditions with respect to applied torque, muscle damage, and any other possible effectors of hypertrophy.

We hypothesized that due to the greater relative stress (i.e. force per active muscle fibre) which is placed on active muscle fibres during high velocity eccentric contractions, training using high velocity (3.66 rad/s) eccentric contractions as opposed to low velocity (0.35 rad/s) eccentric contractions would:

1) Increase the amount of ultrastructural damage seen after a bout of training;
2) Cause greater hypertrophic gains in muscle fibre, and whole muscle CSA;
3) Increase strength gains at all velocities concentrically, but even more so eccentrically; and,
4) Increase the percentage of type IIa fibres and the relative abundance of IIa MHC, while decreasing the proportion of type IIx fibres and the relative abundance of IIx MHC.

Chapter II

Augmented Muscle Hypertrophy with High Velocity Eccentric Resistance Training in Young Males

Introduction

Resistance training results in muscle hypertrophy and strength gains via the addition of myofibrillar proteins resulting in an increase of myofiber CSA (McCall et al., 1996; Widrick et al., 2002), as well as neuromuscular adaptations (Enoka and Fuglevand, 2001). The muscular hypertrophy seen with resistance training is due to chronic increases in muscle protein synthesis that exceed protein breakdown (MacDougall et al., 1995; Phillips et al. 1997). However, transient net gains in muscle protein only occur in the fed state following acute bouts of resistance exercise (Rasmussen et al., 2000; Tipton et al., 1999; Tipton et al., 2001).

Training using only eccentric contractions has been shown to increase muscle hypertrophy more than that of concentric training alone (Hather et al., 1991; Higbie et al., 1996; Hortobágyi et al., 1996a; Hortobágyi et al., 1996b; Hortobágyi et al., 2001). However, the mechanism(s) responsible for the greater hypertrophic response observed during training with eccentric contractions remain unknown. One hypothesis is that eccentric contractions produce a greater stress per active muscle fibre, since fewer motor units are active during an eccentric contraction compared to a concentric contraction of the same magnitude (Grabiner et al., 2002). This larger stress per cross bridge has been shown to result in significant ultrastructural muscle damage after eccentric exercise in both untrained (Gibala et al., 1995) and trained persons (Gibala et al., 2000). The muscle damage caused by eccentric contractions may also cause increased protein synthesis due to a greater response of local growth factors such as IGF-1 (Bamman et al., 2001). Therefore it is feasible that a superior hypertrophic response may be observed in a muscle that is repetitively exposed to greater eccentric-induced muscle damage.

The use of high-velocity eccentric training has also shown to result in increased strength (Paddon-Jones et al., 2001) and possibly hypertrophy of skeletal muscle (Farthing and Chilibeck, 2001). According to the force-velocity curve, eccentric force production increases with increasing contraction velocity, although this relationship has not always been illustrated (Westing et al., 1991). However, motor unit recruitment remains similar for maximal eccentric contractions throughout a range of velocities, since muscle force only acts in an attempt to slow the contraction and does not have to overcome it (Enoka, 1996). Therefore, increased stress may be placed on individual cross bridges due to the greater force generation without the subsequent recruitment of addition motor units. This augmentation of force per cross bridge would then be more likely to increase any mechanical damage that is observed after fast eccentric training.

Higher-velocity eccentric training has also been shown to result in an increase in type IIx fibres, with a subsequent decrease in type I fibre percentage (Paddon-Jones et al., 2001). This slow to fast transition contradicts previous research which indicates that slow to fast fibre transitions are generally only thought to occur following disuse (Andersen et al., 1995; Berg et al., 1997; Hortobágyi, et al., 2000; Talmadge et al., 2002). It has been hypothesized that the increase in type IIx fibres occur due to a preferential recruitment of fast twitch fibres when fast eccentric contractions were implemented (Paddon-Jones et al., 2001).

Although the increase in muscle strength (Paddon-Jones et al., 2001) and hypertrophy (Farthing and Chilibeck, 2001) after high-velocity contractions has been demonstrated to be superior over that attained by low-velocity eccentric contractions, it is unknown whether high versus low velocity eccentric contractions cause different amounts of muscle damage. Therefore the purpose of this study was to investigate whether fast

eccentric contractions would induce greater strength and hypertrophic gains in skeletal muscle, as well as the possibility of greater mechanical damage being induced by the higher velocity eccentric contractions, investigated in a smaller acute exercise study. We hypothesized that due to greater stress per muscle fibre, training with high velocity (3.66 rad/s) as opposed to low velocity (0.35 rad/s) eccentric contractions, would induce a greater amount of ultrastructural damage after a bout of training. Likewise, fast training would induce greater hypertrophic gains seen at both the muscle fibre level and whole muscle CSA, while concurrently increasing strength at all concentric, but more so at eccentric velocities. We also proposed that fast training would increase the percentage of type IIa fibres and the relative abundance of IIa MHC, while decreasing the proportion of type IIx fibres and IIx MHC.

Methods

Experiment I – Training Study

Subjects

Twelve healthy men (age 23.8 ± 3.4 y, height 178.5 ± 9.6 cm, weight 82.5 ± 13.3 kg) who were recreationally active (i.e. no weight training) participated in the 8 week training study. Subjects were required to complete a routine medical screening questionnaire, and based on their responses all were deemed healthy. Subjects were advised of the purposes and risks associated with the study, and gave written informed consent. The project was approved by the Hamilton Health Sciences Corporation Research Ethics Board.

Experimental Protocol

One week prior to the study start date, subjects attended a familiarization session on the testing/training apparatus (Biodex-System 3, Biodex Medical Systems Inc., New York). On the study start date subjects had baseline cross-sectional computerized tomography (CT) scans taken of the midline of the bicep brachii of the right and left arms. Immediately following the CT scans, subjects underwent a series of pre-training (PRE) strength tests (*see Strength Measurements*) on both arms independently. Approximately 24 h later subjects reported back to the Ivor Wynne Centre, and muscle biopsy samples were taken from the belly of the bicep brachii muscle of each arm. Arms were then randomly assigned to be trained using either fast (FAST) or slow (SLOW) eccentric training group.

Subjects commenced training following one week of rest. For the next eight weeks subjects reported to the Ivor Wynne Centre every Monday, Wednesday and Friday for exercise training. During the first week 1 set (x 10 repetitions) was completed for both FAST and SLOW arms, and every week subsequent an additional set was added to a maximum of 4, with 120 s of rest between each set. For the remaining 4 weeks, subjects continued to complete 4 sets on every training day.

Following the 8 week training protocol, subjects were again given 1 week of rest prior to post-training (POST) testing. CT scans were again taken from the same position of each arm, and subsequently post-training strength measurements were recorded in the same manner as the pre-training tests. Post-training muscle biopsies were then obtained from a position approximately 5 cm superior to the pre-training biopsies (Fig. 2).

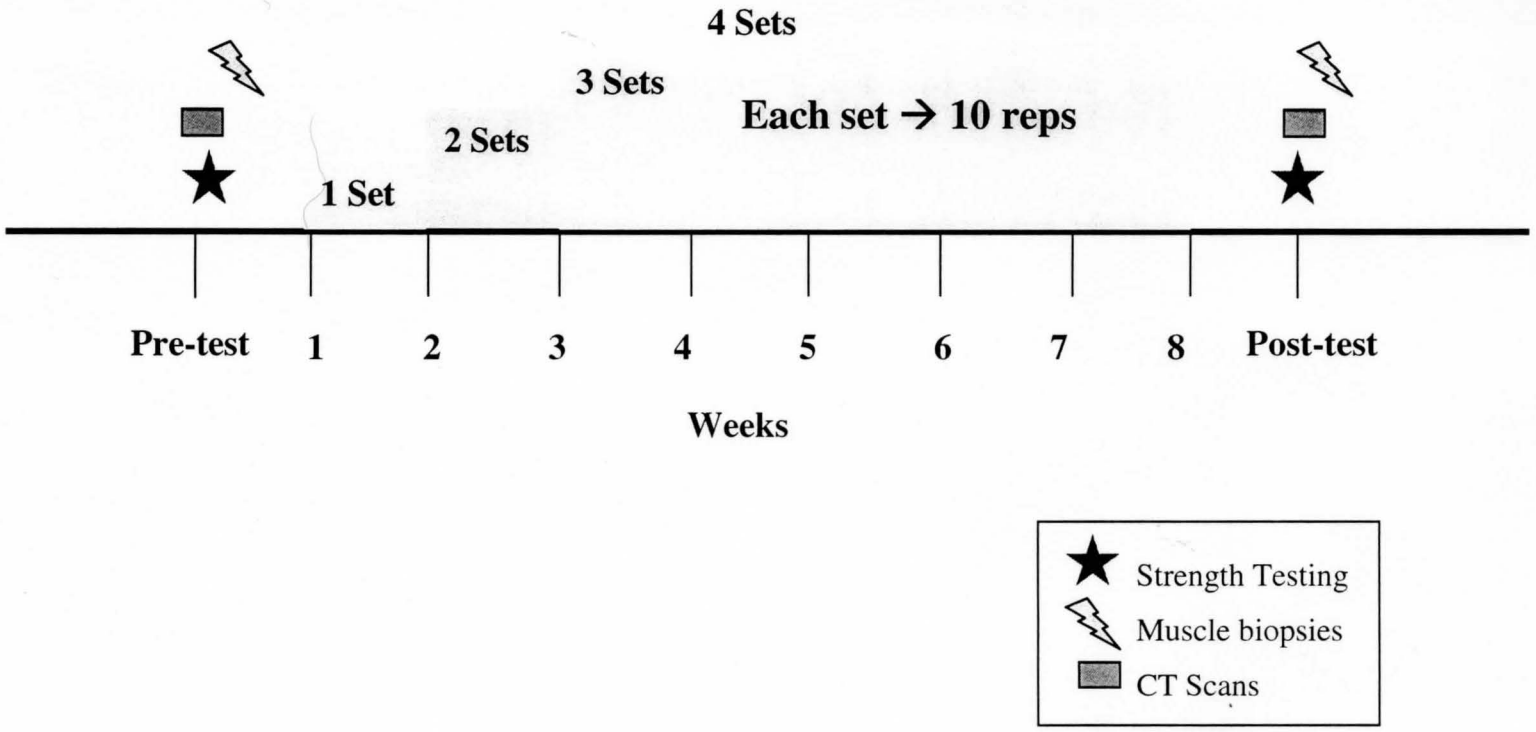

Figure 2. Outline of study design.

Strength Measurements

Strength tests were performed in a randomized order at various contraction speeds (0.35 rad/s, 1.05 rad/s, 2.10 rad/s, 3.14 rad/s, and 3.66 rad/s) and types (eccentric, concentric and isometric). Subjects placed their elbow on a positioning pad so that the ulnar-humeral joint was at the axis of rotation of the Biodex machine, and they could comfortably grasp the lever arm handle, while their forearm was in a supinated position. A restraining strap was placed diagonally over the involved shoulder to limit involvement of other muscle groups.

Isometric Torque

Subjects performed three repetitions of a maximum voluntary contraction (MVC) at 1.05 rad (120°) of elbow flexion. Each contraction was 5 s in duration with 30 s of rest between contractions. Maximum isometric torque was considered to be the highest peak value of the three contractions.

Concentric Torque

Concentric torque was recorded as the highest peak torque of three repetitions through 1.57 rad (90°) of motion. Every repetition began at 3.05 rad (175°) of elbow flexion and concluded when the subject flexed his arm, and the Biodex lever arm, with maximal force to 1.48 rad (85°) of elbow flexion. Concentric torque was evaluated at the five different velocities.

Eccentric Torque

Measurements of Eccentric torque were made at the five testing velocities in a similar manner as for concentric torque. Eccentric torque was taken from the highest peak torque of three repetitions starting at 1.48 rad (85°) and concluding at 3.05 rad (175°) of elbow flexion. Each eccentric contraction was completed by the subject maximally resisting the lever arm which was returning to the resting position.

CT Scans

The midline of the belly of the bicep brachii was determined by measuring half way between midpoint of the antecubital and axilla areas. The arm was then inserted into a small cylinder which functions as the measurement area of the peripheral quantitative computer tomography (pQCT) densitometer (Stratec XCT 2000 Bone Densiometer, Norland Medical Systems, Inc., Connecticut). The subject's arm remained motionless for approximately 4 minutes while the scan was performed. Scans were then sectioned into bone, muscle, and fat mass according to the bundled densitometer software. Previous studies (Rittweger et al. 2000) have reported the coefficient of variation of multiple muscle scans on the XCT 2000 as being 1.15% between trials. In our laboratory, repeated scans of the midline of the biceps had less than 3% variation.

Muscle Biopsies

Needle biopsy samples were obtained from each subject under local anesthesia (2% lidocaine) using manual suction. One biopsy was taken from the medial portion of the right and left bicep brachii after the PRE strength testing session to establish a baseline measurement. Another biopsy sample was taken from each of the trained arms in the same

manner POST. Samples were immediately dissected free of fat and connective tissue, and placed in optimum cutting temperature (OCT, Tissue Tech^{TM}) embedding medium with its fibres oriented perpendicular to the plane in which it was to be cut. The samples were then quick frozen in isopentane cooled by liquid nitrogen, and stored at -50°C until subsequent analysis.

Histochemical Analysis

The frozen OCT mounted muscle samples were serially cross-sectioned to 10μm thick on a Microtome cryostat (Model HM500OM, MICROM International, Waldorf, Germany) for histochemical analysis. Myofibrillar adenosine triphosphate (mATPase) histochemistry was performed using preincubation pH value 4.60 (Brooke & Kaiser, 1970) (50mM potassium acetate, 17.5mM calcium chloride) for 6.5min to determine muscle fibre type composition. Slides were then rinsed with distilled water and incubated in 3mM ATP using an alkaline solution (75mM glycine, 40.5mM calcium chloride, 75mM NaCl, 67.5mM NaOH, adjusted to pH 9.4) for 45min 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 and the samples were incubated in 1% $CaCl_2$ for 3min at room temperature. Slides were again rinsed with distilled water and incubated in 2% $CoCl_2$ for 3min at room temperature. Another rinse with distilled water and an incubation in 1% ammonium sulphide for 1min at room temperature followed. Samples were rinsed with distilled water five times before being dehydrated by incubating for 2min in each ethanol concentrations (70, 80, 90, 95 and 100%). Samples were then cleared using

xylene. After the slides were dried, coverslips were mounted using Permount (Fisher SP15) and allowed to dry overnight.

Sections were viewed under 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 by using SPOT software (V3.2.4 for Windows, SPOT Diagnostic Instruments, Inc.), Image-J software (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 three and five, and largely dependent on the quality of the serial sections. Each image contained approximately 30-50 fibres. Three fibres (type I, IIa and IIx) were distinguished using the Image-J software by setting cut-off limits resulting in the creation of optical density 'bins' according to the darkest (type I), lightest (type IIa) and intermediate (type IIx) 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 IIx 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 optical density bins were created that were: 0-95 for dark areas, 100-175 for intermediate areas, 180-255 for light areas. Using these cutoffs 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.

MHC Protocol

Mixed muscle MHC analysis was carried out as described previously (Staron et al., 2000). Briefly, four to six serial sections from the OCT-embedded muscle sample were cut (20μm) and placed into microfuge tubes containing 250μl of lysing buffer (10% wt/vol glycerol, 5% vol/vol 2-mercaptoethanol, and 2.3% wt/vol sodium dodecyl sulphate (SDS) in 62.5mM Tris (hydroxymethol) aminomethane, pH 6.8) and were heated for 10min at 60°C. Approximately 4-6μl of the lysed muscle extract was loaded into a 20cm x 20cm x 1.5mm SDS polyacrylamide gel, with pre- and post-training samples adjacent to one another. The gel was poured and set in such a way that the top 25% of the gel was a 4% stacking gel, whereas the remaining 75% of the gel was a 4-8% acrylamide gradient. Samples were run overnight (19-21h) at 120V and subsequently stained with Coomasie Blue. Three separate and distinct MHC isoforms (I, IIa, and IIx) were visually identified according to their masses (as compared with known molecular weight standards). The gels were then scanned using a laser densitometer and the relative staining intensity (i.e., number of arbitrary densitometric units) of each band was calculated and the intensity was expressed as a percentage of the total staining intensity (i.e., the summed arbitrary units of all three bands).

Statistical Analysis

Fibre size, fibre type, and MHC data were analyzed using a two-factor repeated measures analysis of variance (ANOVA), with time (PRE versus POST), and condition (FAST versus SLOW) as the within factors. Strength data was analyzed using a four-factor repeated measures ANOVA, with time (PRE versus POST), condition (FAST versus SLOW), contraction (ECC versus CON), and velocity (5 levels - 0.35 rad/s, 1.05 rad/s,

2.10 rad/s, 3.14 rad/s, and 3.66 rad/s) as the within factors. Statistical significance for all analyses was accepted as $P \leq 0.05$. Significant main effects and interactions were further analyzed using a Tukey honestly significant difference post hoc test. Values presented are means ± SEM.

Experiment II – Acute Study

Subjects

Seven males (Age 21.7 ± 2.4 yr, height 178.9 ± 8.1 cm, weight 79.1 ± 7.1 kg) who were recreationally active (i.e. no weight training and no more than 3 exercise bouts per week) participated in the acute study training protocol. Subjects were required to complete a routine medical screening questionnaire, and based on their responses were deemed healthy. All subjects were advised of the purpose of determining the difference in muscle damage between fast and slow eccentric contractions, the risks associated with the study, and consequently gave written informed consent. The project was approved by the Hamilton Health Sciences Corporation Research Ethics Board.

Experimental Protocol

For one week prior to subjects reporting to the Ivor Wynne Centre for testing, subjects were instructed to refrain from strenuous upper body activity. The first study day subjects were questioned for arm dominance and had a muscle biopsy removed from the belly of bicep brachii of both right and left arms. Subjects also received instruction on the exercise protocol and use of the Biodex apparatus (Biodex-System 3, Biodex Medical Systems Inc., New York), which would be used for testing. For the week following the first biopsy, subjects were again asked to refrain from strenuous upper body activity.

Upon arrival to the Ivor Wynne Centre subjects completed the twitch protocol, then were given 10 minutes of rest and were then prepared for the exercise protocol. The following day subjects returned to the Ivor Wynne Centre to repeat the twitch protocol and subsequently had the second biopsy sample from each arm removed. Subjects again returned to repeat the twitch protocol 24 and 72 hrs after the second biopsy session.

Exercise Protocol

The exercise protocol was completed on the Biodex apparatus (Biodex-System 3, Biodex Medical Systems Inc., New York). Subjects placed their elbow on the Biodex positioning pad so that their forearm was in a supinated position, the ulnar-humeral joint was at the axis of rotation of the Biodex lever arm, and they could comfortably grasp the lever arm handle. A restraining strap was placed diagonally over the involved shoulder to limit involvement from other muscle groups. Each subject completed 3 sets (10 repetitions) of maximal eccentric contractions throughout 1.57 rad (90°) of arm flexion with 120 seconds of rest between sets. One arm was trained at a fast velocity (3.66 rad/s) while the other was trained at a slow velocity (0.35 rad/s).

Twitch Protocol

Evoked twitch data was gathered at 0 h, 24 h, 48 h, and 96 h post exercise. Subjects were seated in a height adjustable chair and positioned so that the involved arm could be placed comfortably over a preacher curl weight bench. Subjects were then restrained over the involved shoulder using a seat belt attached diagonally down towards the opposite hip. Subjects' involved arm was then tightly strapped to a hinged, padded board disallowing any movement, with forearm supinated and elbow flexed at 1.57 rad

(90°). The board was attached to a torque measuring device and remained stationary to measure only isometric torque. Subjects' involved bicep brachii were prepared and had one positive and one negative electrode placed over the belly of the bicep, and a ground electrode on the olecranon process of the ulna. Subjects were then instructed to remain motionless and relaxed while the bicep brachia was repeatedly shocked until maximum torque was achieved with minimum voltage. The highest torque was recorded. A MVC was then completed for 10 seconds with an interpolated twitch given at 3 seconds. After the 10 s MVC another twitch was given. The same procedure was then completed with the opposite arm. Peak torque was recorded on the pre-MVC twitch, MVC, post-MVC twitch, and the MVC interpolated twitch. Time to peak was recorded for the pre- and post-MVC twitches. All values were recorded for each arm of each subject.

Muscle Sampling

Needle biopsy samples were obtained from each subject under local anesthesia (2% lidocaine) using manual suction. One biopsy was taken from the medial portion of the right and left bicep brachii after the pre-training strength testing session to establish a baseline measurement. Another biopsy sample was taken from each of the trained arms in the same manner 24 hrs after the 3-set training protocol was completed. Samples were immediately dissected free of fat and connective tissue, and dissected into two portions. The larger portion was placed in optimum cutting temperature (OCT, Tissue Tech^{TM}) embedding medium with its fibres perpendicular to the plane in which it was to be cut. The samples were then quick frozen in isopentane cooled by liquid nitrogen, and stored at -50°C for future analysis. The smaller portion was placed into a chilled (4°C) fixative (2%

gluteraldehyde buffered with 0.1% sodium cacodylate) for staining with toluidine blue as described previously (Beaton et al., 2002).

Light Microscopy

After initial fixation, the tissue samples were postfixed in osmium tetroxide, dehydrated in graded baths of ethyl alcohol, and embedded in an epoxy resin (Spurr's) with the fibres oriented longitudinally. Each block was then sectioned (0.5 μm) and stained with toluidine blue.

Individual fibres from each longitudinal muscle section were studied under 1000x magnification and examined for moderate (3-10 continuous and/or adjacent Z-bands) and extreme (10 or more continuous and/or adjacent Z-bands) Z-band streaming (Gibala et al., 1995). Sample areas were calculated and the extent of Z-band streaming was expressed per mm^2 of muscle. All muscle sections were scored and viewed blind to the investigator as to the treatment (fast or slow training) and subject. Using this method it has been shown that sections identified as having Z-band streaming, were shown also to have disrupted Z-bands using electron microscopy (Beaton et al., 2002; Stupka et al., 2000).

Statistical Analysis

Twitch data were analyzed using a two-factor repeated measures analysis of variance (ANOVA), with time (4 levels – 0 hr, 24 hr, 48 hr, and 96 hr), and velocity (FAST versus SLOW) as the within factors. Significant main effects and interactions were further analyzed using a Tukey honestly significant difference post hoc test. Statistical significance for all analyses was accepted as $P \leq 0.05$. Values presented are means ± SEM.

Results

Experiment I – Training Study

Strength Measurements

Subjects generated higher maximal torques using eccentric contractions when compared to concentric contractions ($P<0.05$, main effect for contraction) following training. Higher contraction velocities produced significantly higher torques when compared to slower velocities except when 3.14 rad/s was compared to 2.10 rad/s ($P<0.05$, main effect for velocity). Fast training increased maximum torques an average of 10.3±16.4 Nm, whereas slow training only increased strength by 7.3±15.0 Nm. ($P<0.05$, time x condition interaction; Fig. 3). Peak torques decreased significantly after the first training session and reached the lowest point at day 6 ($P<0.05$, main effect for time; Fig. 4). After session 6, torques continued to rise throughout the training sessions, but never increased significantly more than training session 1. Fast arms produced significantly higher peak torques than that of slow arms ($P<0.05$, main effect for condition).

CT Scans

Whole upper-arm CSA increased significantly after 8 weeks of eccentric biceps training as measured by a peripheral CT scan ($P<0.05$; main effect for time; Fig. 5). Although not statistically significant, a trend ($P=0.065$) was demonstrated in whole muscle hypertrophy in the fast versus the slow arm with the fast arms increasing in CSA more then the slow arms. The fast trained arms had an average increase of 6.8±5.5 % whereas the slow arms only had an average increase CSA of 5.1±5.7 % ($P=0.065$, time x condition interaction).

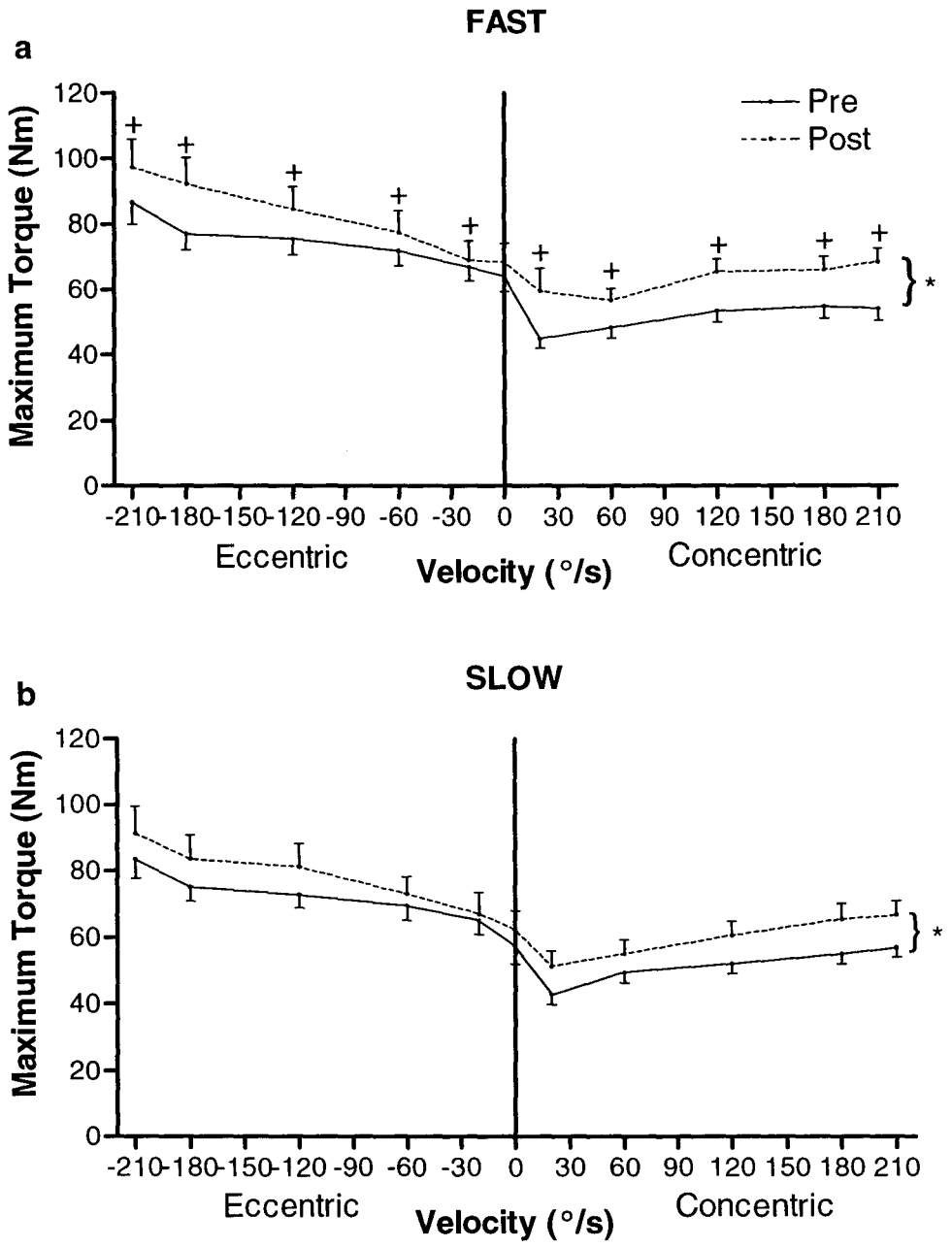

Fig. 3 Peak Torque before and after 8-weeks of Fast and Slow training. Fast arm – a. Slow arm – b. * Significant main effect for time ($P<0.05$). + Significant time x condition interaction at all testing velocities ($P<0.05$). Values are means±SEM ($N=12$).

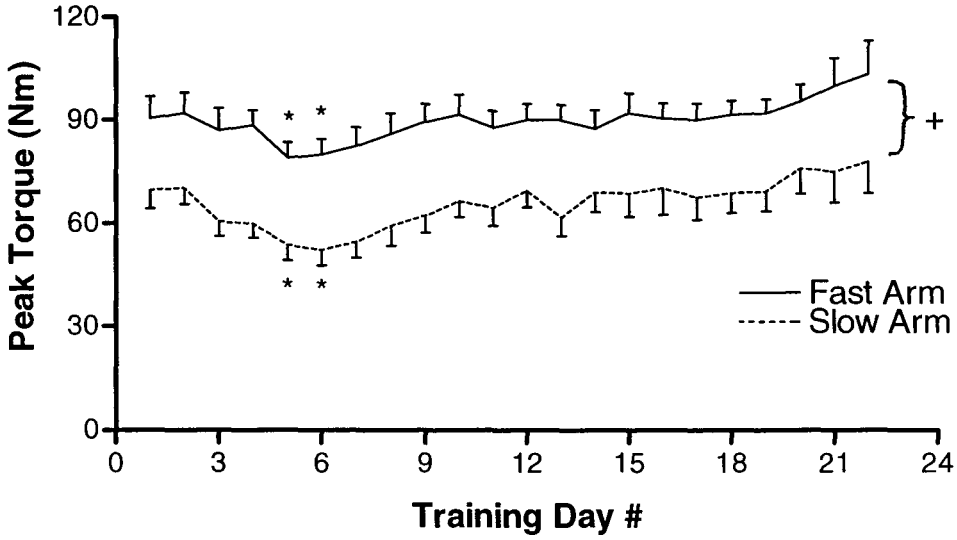

Fig. 4 Daily peak torque values for subjects Fast and Slow arms. * Significantly different from pre values within the same arm (P<0.05). + Significantly different from SLOW arm at the same time. Values are means±SEM (N=12).

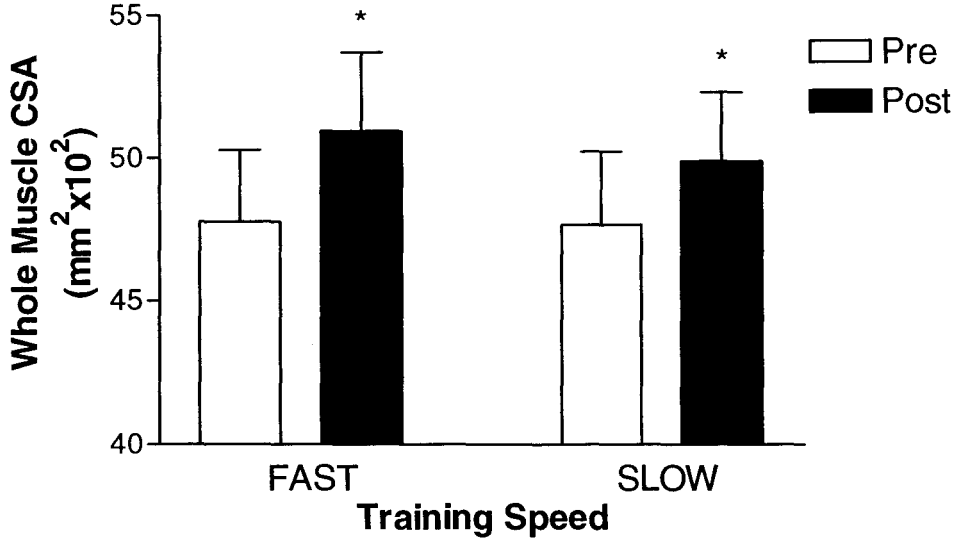

Fig. 5 Whole muscle CT scan CSA before and after 8-weeks of Fast and Slow training. * Significant main effect for time (P<0.05). Time x condition interaction P=0.065. Values are means±SEM (N=12).

Muscle Fibre Size

Training significantly increased the mean CSA of type I muscle fibres ($P<0.05$, main effect for time; Fig. 6) an average of 9.3±12.0%. A significant difference was also detected between the fast and slow training for both type IIa and IIx fibres ($P<0.05$, time x condition interaction). Post hoc analysis revealed that type IIa fibres increased significantly after fast training, but did not increase significantly after slow training. There was however a significant difference between baseline values of the slow and fast arms in both type II fibre classifications. Type IIx fibres had significant hypertrophy after both training conditions; however, fast training induced significantly more hypertrophy than did slow training. Overall, fast training induced hypertrophy more than slow training for type IIa ($P<0.05$) and IIx ($P<0.05$), with a trend toward a greater increase in type I fibres ($P=0.053$), according to paired t-tests, based on changes (Fig. 7).

Muscle Fibre Type

A significant decrease was observed in the percent distribution of type IIx fibres ($P<0.05$, main effect for time; Fig. 8). Although statistical significance was not reached, a very strong trend was also observed towards a decrease in the percent area that type IIx fibres occupy ($P=0.056$; Fig. 9). There was, however, a significant difference in the area of type IIa fibres ($P<0.05$, time-condition interaction). Post hoc analysis revealed that type IIa fibres increased total area significantly more after fast training (8.4±8.6%) when compared to slow training (1.7±10.9%).

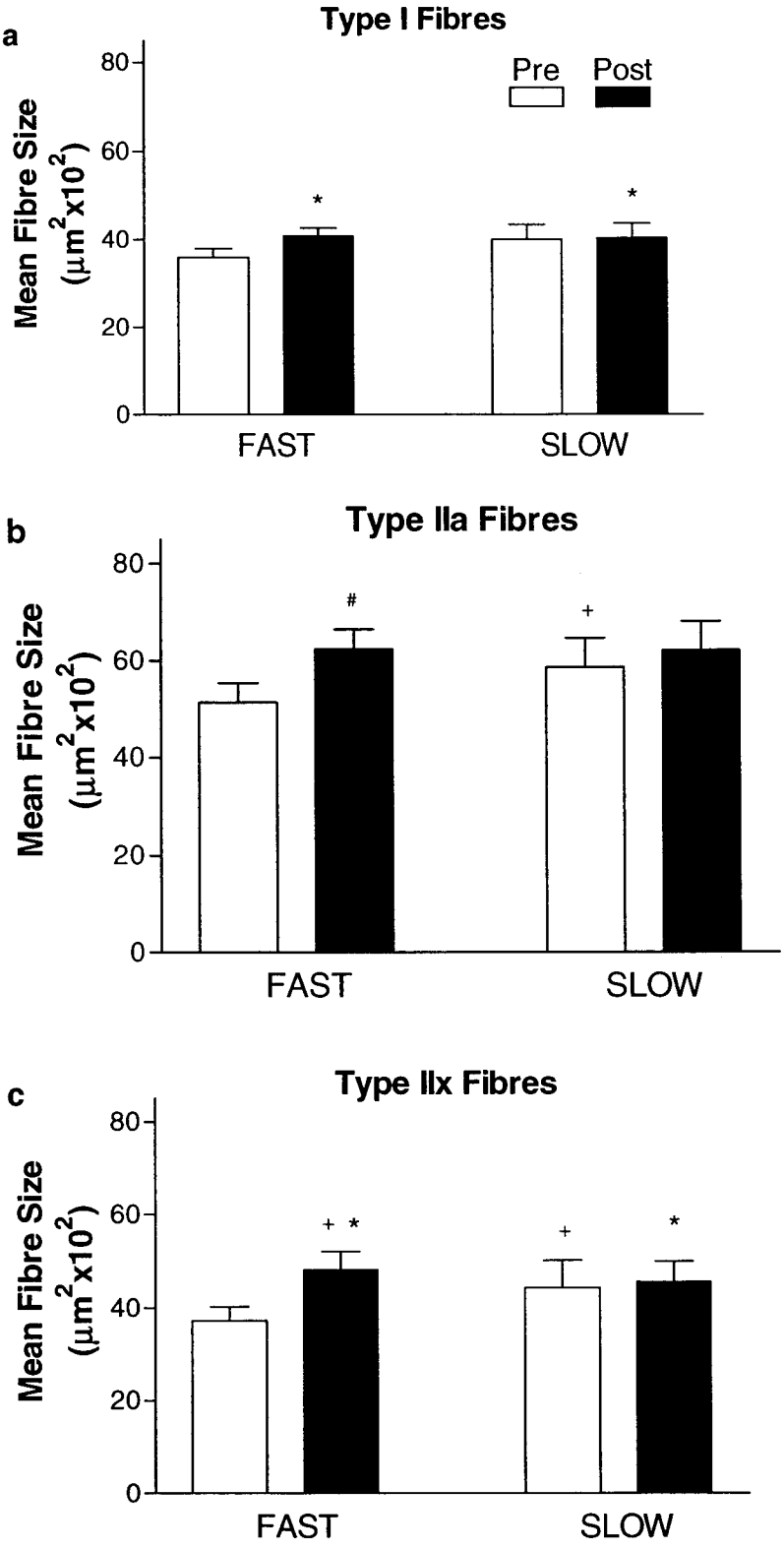

Fig. 6 Muscle fibre size adaptations before and after 8-weeks of Fast and Slow training. Type I fibres – a. Type IIa fibres – b. Type IIx fibres – c. * Significant main effect for time ($P<0.05$). # Significantly greater than pre value of the same arm ($P<0.05$). + Significantly different from SLOW arm at the same time ($P<0.05$). Values are means±SEM (N=11).

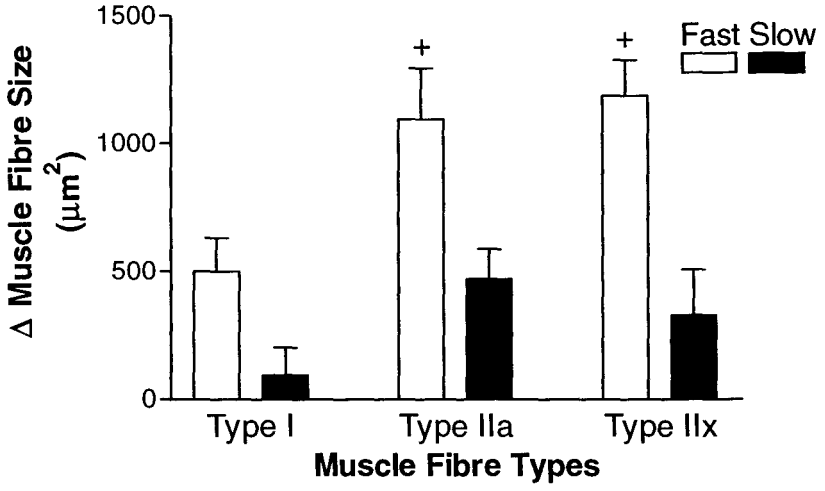

Fig. 7 Muscle fibre size differences before and after 8-weeks of Fast and Slow training. + Significantly different from SLOW arm at the same time ($P<0.05$). Values are means±SEM (N=11).

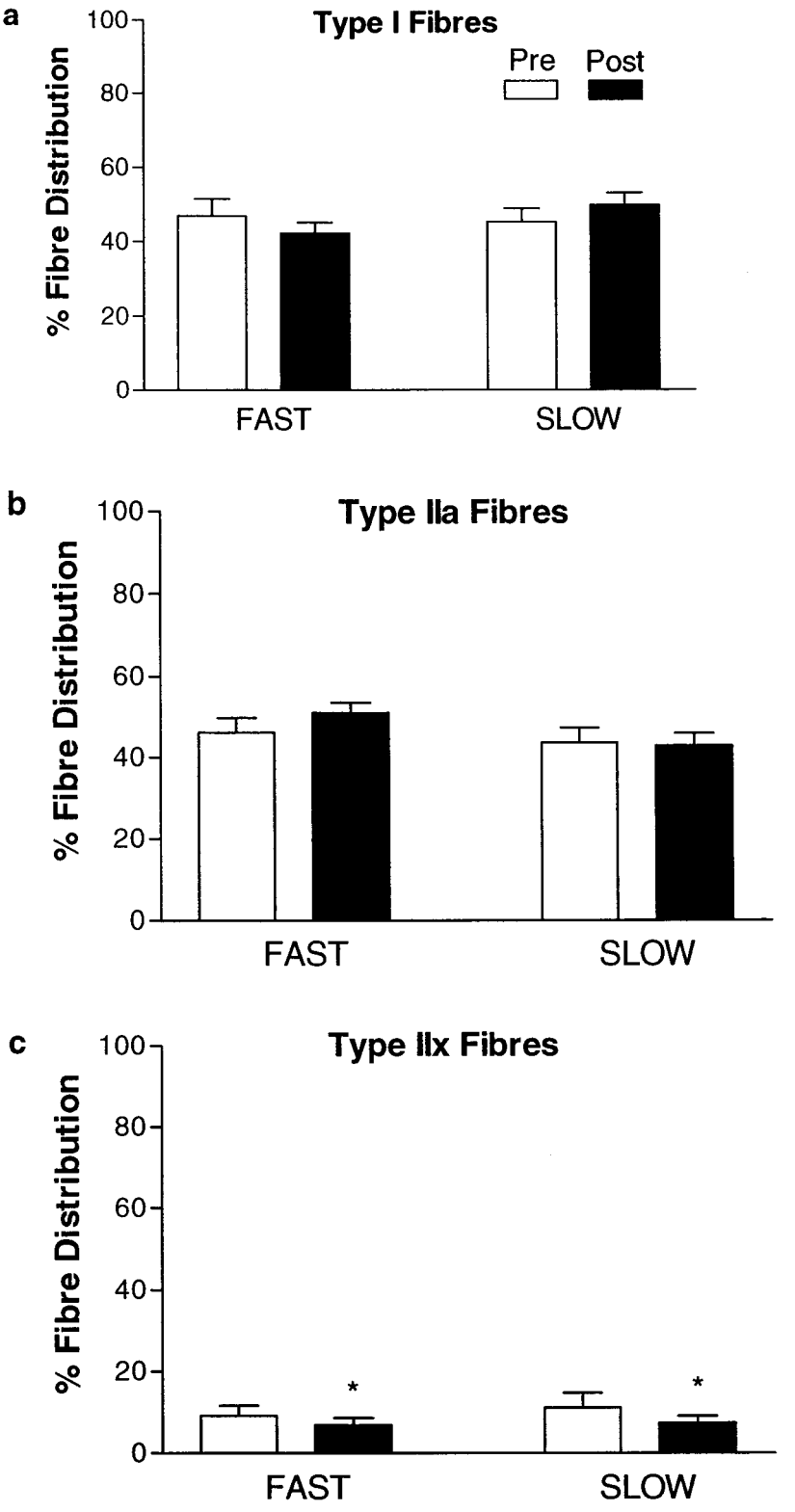

Fig. 8 Fibre type percent distributions before and after 8-weeks of Fast and Slow training. Type I fibres – a. Type IIa fibres – b. Type IIx fibres – c. * Significant main effect for time ($P<0.05$) within the same group. Values are means±SEM (N=12).

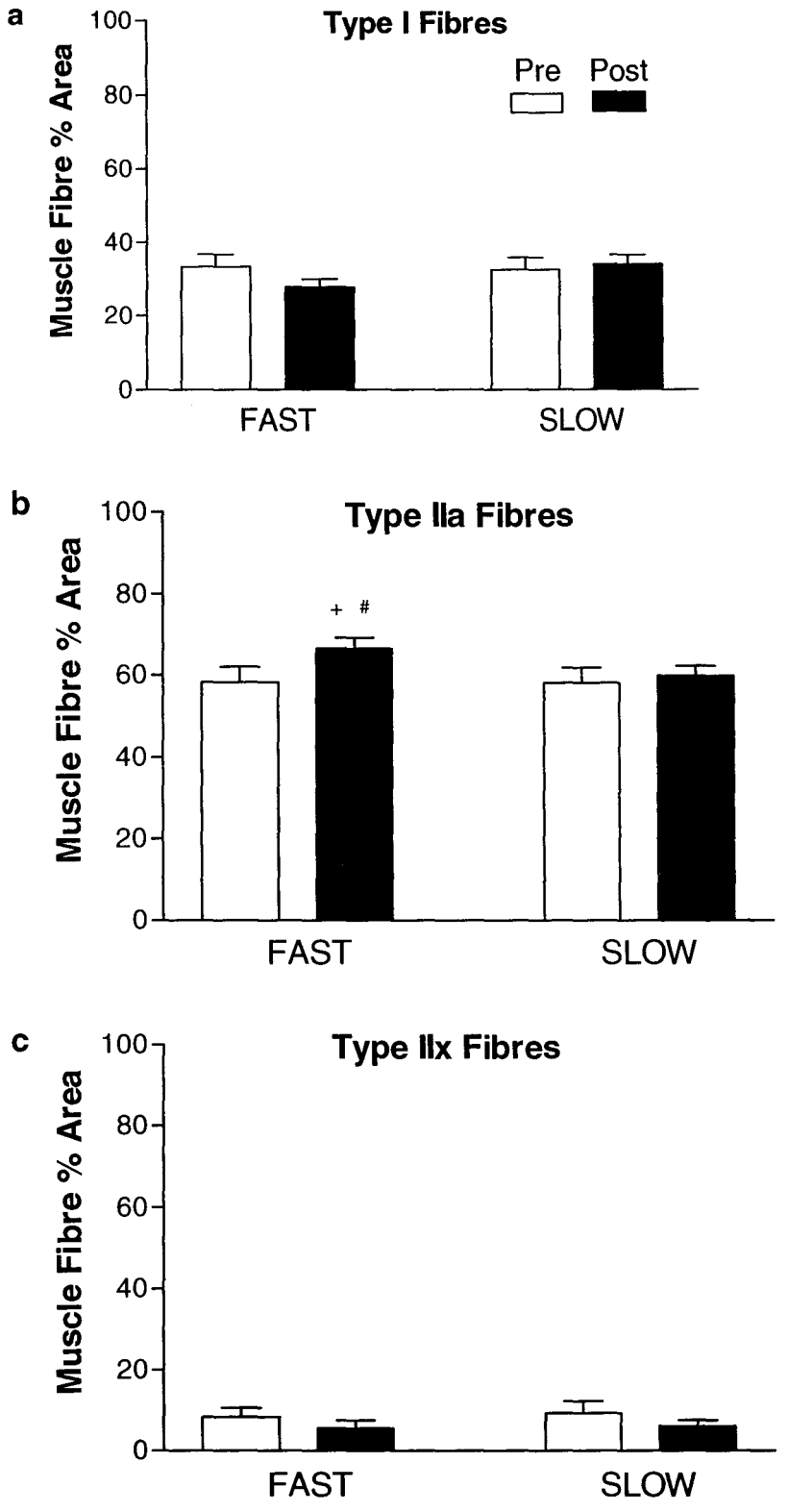

Fig. 9 Fibre type percent area before and after 8-weeks of Fast and Slow training. Type I fibres – a. Type IIa fibres – b. Type IIx fibres – c. # Significantly different from pre value of the same arm ($P<0.05$). + Significantly different from SLOW arm at the same time. Values are means±SEM ($N=12$).

MHC Content

The percentage of MHC isoforms expressed did not change to a differential degree between training conditions. There was, however, a significant decrease in the percentage of type IIx isoform expressed in both fast and slow arms after training ($P<0.05$, main effect for time; Fig. 11). Similarly, there was a trend towards an increase in the percentage of type IIa isoform present after training ($P=0.08$, main effect for time). The percentage of MHC isoforms expressed according to gel electrophoresis correlated well with the percent area of each fibre type as analyzed by ATPase histochemistry ($r = 0.95$, $P<0.0001$; Fig. 10).

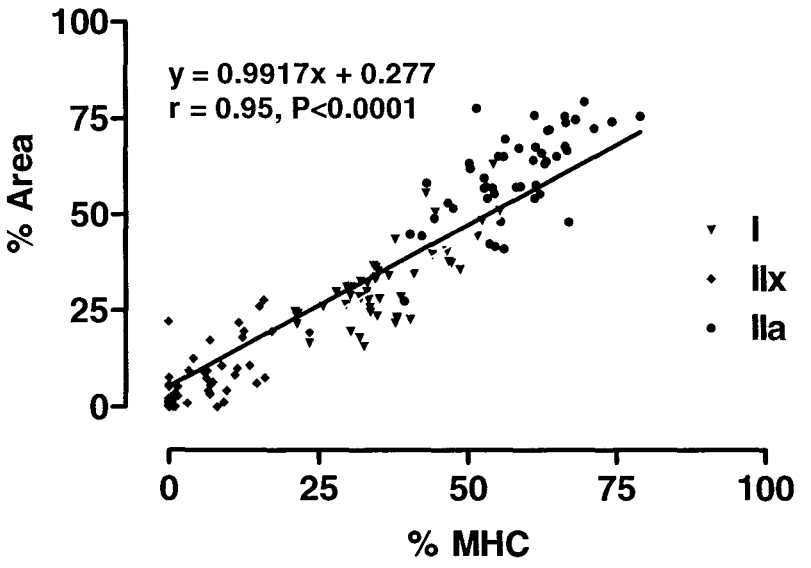

Fig. 10 MHC isoform vs. percent area distributions (N=12).

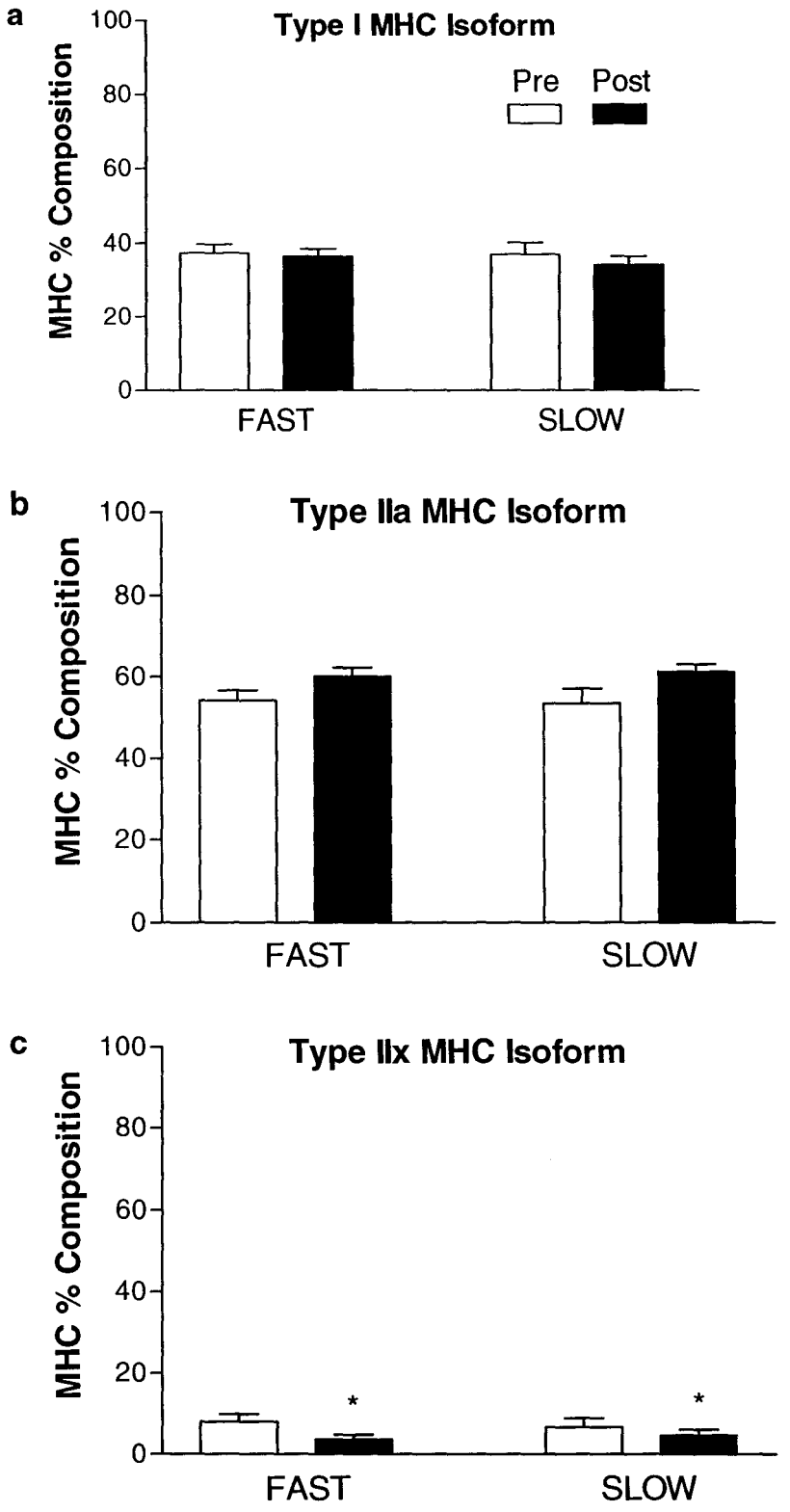

Fig. 11 MHC isoform distributions before and after 8-weeks of Fast and Slow training. Type I fibres – a. Type IIa fibres – b. Type IIx fibres – c. * Significant main effect for time ($P<0.05$). Values are means±SEM (N=12).

Experiment II – Acute Study
Evoked Twitch Characteristics

A significant decrease was observed for peak MVC after the exercise protocol ($P<0.05$, main effect for time, Table 1). Post hoc analysis revealed that there was a significant decrease in peak MVC torque production between 0 hr and 24 hr ($P<0.05$), with a trend towards a 0 hr to 48 hr decrease ($P=0.079$). There was also a significant difference in the pre-MVC twitch (twitch 1) after the damage protocol ($P<0.05$ main effect for time; Table 1). A significant decrease was seen from 0 hr to 24 hr and 96 hr ($P<0.05$) with a trend towards a 0 hr to 48 hr decrease ($P=0.09$). Likewise, post-MVC twitch (twitch 2) torque decreased after the damage protocol ($P<0.05$, main effect for time; Table 1). A significant decrease was detected between 0 hr and 24 hr, 48 hr and 96 hr ($P<0.05$). There was also a significant decrease between 48 hr and 96 hr ($P<0.05$). Although a significant reduction in torque was seen after the exercise sessions, there was no difference between the fast and slow treatments. No other values including time to peak and total motor unit recruitment was altered significantly with either the fast or slow exercise bouts.

Muscle Damage

The extent of moderate Z-band disruption (expressed per mm^2 of muscle) seen after the fast exercise protocol was significantly more than that seen after the slow exercise protocol ($P<0.05$; Fig. 12). The extent of extreme Z-band streaming was not significantly different ($P>0.05$) between fast and slow exercised arms, however, the fast exercised arm showed 0.09 ± 0.15 areas of extreme damage per mm^2 of muscle, whereas the slow arm only showed 0.04 ± 0.11 per mm^2.

Measure	Speed	0 HR	24 HR	48 HR	96 HR
MVC (Nm)	Fast	72.6±5.4	62.2±6.1*	67.8±4.4	69.3±3.4
	Slow	68.1±4.8	58.1±6.5*	57.6±6.2	59.9±5.8
MUA (%)	Fast	98.2±2.0	98.6±1.0	98.3±1.6	98.8±0.7
	Slow	98.9±1.5	99.0±0.5	98.1±1.9	98.5±1.5
PTT-1 (Nm)	Fast	11.6±0.8	9.2±1.1*	10.4±1.2	9.0±1.0*
	Slow	11.1±0.8	7.5±1.3*	8.2±1.7	7.4±1.6*
PTT-2 (Nm)	Fast	16.1±1.3	13.2±1.5*	13.9±1.2*	11.4±1.3*+
	Slow	15.4±1.2	10.5±2.0*	11.9±1.9*	10.2±1.6*+

Table 1 Twitch measurements 0 – 96 h after damage protocol. PTT – Peak Twitch Torque. MUA – Motor Unit Activation. * Significant main effect for time compared to 0 hr ($P<0.05$). + Significantly different from 48 hr. Values are means±SEM (N=7).

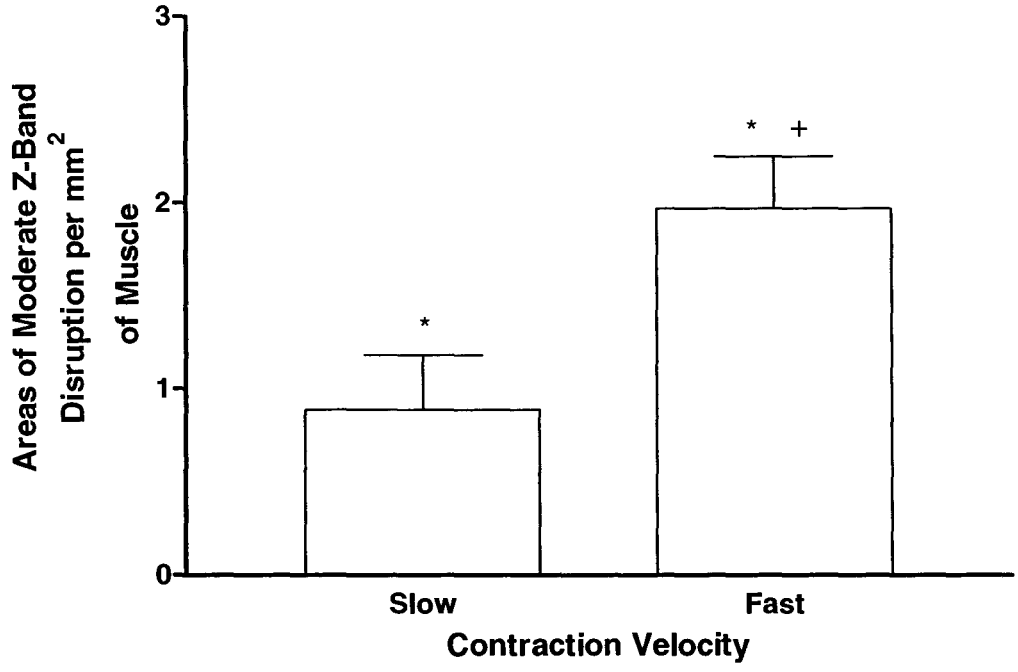

Fig. 12 Z-band disruption seen within biopsy samples 24 h after exercise protocol (see methods for details). * Significantly different from baseline. + Significantly different from SLOW arm at the same time. Values are means±SEM (N=7).

Discussion

The results of the present investigation indicate that fast eccentric training increases the hypertrophic response in skeletal muscle to a greater extent than that of slow eccentric training. This conclusion is supported by a significantly greater increase in muscle fibre size as indicated by ATPase histochemical analysis. Additional support for the greater hypertrophy observed with fast eccentric training was demonstrated by our data measuring the CSA of the midline of the biceps brachii by pQCT. While we did not observe a significant difference between the degree of change in the fast and slow arms, there was a convincing trend (P=0.065) towards the fast arm having greater hypertrophy over the slow trained arms. Although muscle CT scans have been shown to have a very high reproducibility across days (Rittweger et al., 2000), it is possible that scans could have been made at slightly different anatomical locations, introducing enough measurement error to obscure differential changes between the fast and slow arms. Due to the limited number of subjects and some pre-to-post scan variability, it is possible that we have made a type II statistical error.

The use of ATPase histochemical staining has been used extensively to determine muscle fibre hypertrophy (Hather et al., 1991; Hortobágyi et al., 1996a; Lexell et al., 1993; Mayhew et al., 1995). Histochemical staining and fibre CSA measurement is, however, somewhat of a variable measurement tool, and is dependent upon the number of fibres which can accurately be counted (McCall et al., 1998; McGuigan et al., 2002). McCall et al. (1998) reported that 50 fibres from each major fibre type were sufficient to characterize type I and II fibre CSA of the biceps brachii, however, fibre subtypes were not assessed. Further analysis by McGuigan et al., (2002) determined that 50 fibres were sufficient to determine type IIx fibres, but 150 and 200 fibres respectively were required to get accurate

estimates of CSA from type I and type IIa fibres respectively, due to the greater inter-fibre variability in size in these subpopulations. The present study did reach the previously stated minimum fibre number according to McCall et al. (1998), however sufficient fibre numbers could not be reported from the current biopsy samples according to McGuigan et al., (2002). For two reasons we believe that the results of our analysis of CSA are valid. First, taken together the increases in fibre CSA, across all fibre types, agreed with the changes we observed in whole muscle CSA. Also, the greater variability in fibre size in type I and IIa fibres would make it more, not less, difficult to observe significant increases in fibre area. In other words, the likelihood of making a type I error is lower.

As seen previously (Hather et al., 1991; Hortobágyi et al., 1996a; Lexell et al., 1993; Mayhew et al., 1995), greater type II fibre hypertrophy was seen in the present study. The most likely explanation for this repeatable outcome is due to the pattern of fibre type recruitment. The size principle states that smaller, less-fatigable motor units are recruited first, and larger type II motor units are sequentially recruited as needed with increasing force. However, evidence has been published indicating that eccentric contractions follow a 'compressed' size principle recruitment pattern and as a consequence eccentric contractions result in a preferential recruitment of fast twitch motor units (Nardone et al., 1989). A preference for type II motor unit recruitment would instigate greater muscle damage in those fibres, possibly giving way to a greater hypertrophic response.

Greater structural muscle damage could be one possible mechanism for the increased hypertrophy seen with high velocity eccentric training. Morgan and Allen (1999) hypothesized that when a muscle is lengthened suddenly or with high force, some of the sarcomeres are stretched too far and generate damage. When a muscle is lengthened

slowly and with increasing tension the sarcomeres lengthen uniformly and minimize muscle damage (Morgan and Allen, 1999). Hence, faster lengthening (i.e. high velocity eccentric contractions) may result in greater muscle damage. In the present study, after an acute bout of either fast or slow eccentric exercise, a greater proportion of moderate Z-band streaming was found in fast trained arms as opposed to slow trained arms. It has been demonstrated that the measurement of Z-band streaming using light microscopy in muscle samples has a high degree of inter-site variability, increasing the difficulty of observing true differences between biopsy samples (Beaton et al., 2002a). Given this high variability (Beaton et al., 2002a) it is impressive that we were able to observe a significant difference in damage between the two conditions (Fig. 12).

Increasing the amount of ultrastructural muscle damage would cause a greater response of the muscle regenerative processes including neutrophil and macrophage activity to phagocytose injured muscle. Phagocytic cell invasion has also been associated with the regulation of muscle satellite cells, possibly functioning as the key signaling mechanism for their arrival (Best and Hunter, 2000; Cantini et al., 1994). Therefore, increasing the amount of ultrastructural damage could function to increase the number of satellite cells which migrate to the injured muscle making more nuclei available for the addition to skeletal muscle. Likewise, muscle IGF-1, which has been implicated in increasing muscle protein synthesis (Fryburg et al., 1995), as well as the mediation of satellite cell proliferation (Chambers and McDermott, 1996), has an increased mRNA abundance after eccentric contractions versus concentric contractions (Bamman et al., 2001). Although it has not been confirmed, it is likely that eccentric contractions result in an increase in muscle protein synthesis, as a result of increased muscle IGF-1 mRNA content. If eccentric contractions do result in a greater increase in protein synthesis, it is

possible that fast eccentric contractions could cause a synergistic effect in promoting muscle hypertrophy by increasing satellite cell presence, due to greater muscle damage, thus allowing greater amounts of protein and myonuclei to be added to skeletal muscle.

In the present study muscle damage seen within biopsy samples did cause significant MVC and maximum evoked twitch torque force decrements at 24 hr, however, greater damage in the fast arms did not translate into a greater force reduction after exercise, although this may not have been surprising (Warren et al., 2001). One possible explanation for a lack of greater force reduction with greater Z-band streaming is that structural damage decreases force to a certain extent, but additional muscle damage does not have a large impact on subsequent force decrements. According to Warren et al. (2001), the drop in maximal torque generating capacity seen after high force muscle lengthening is due primarily to the disruption of the E-C coupling process and is not due to the physical disruption of force generating structures. Although not significant, there did seem to be a trend towards a slower recovery of maximal torque production after slow training when compared to fast training, which may indicate greater disruption of the E-C coupling process.

Training increased muscle strength at all tested velocities; however, the overall gains were greater in the fast trained arm. This expected result is most likely the consequence of the greater hypertrophy seen within the fast trained arms. According to previous literature (Higbie et al., 1996; Hortobágyi et al., 1996a; Hortobágyi et al., 1996b), however, strength gains are typically dependent on training modes. Hence, when training with eccentric-only muscle contractions, it is expected that eccentric strength would increase more then concentric strength. After the 8-week eccentric training protocol, maximum force generation was not significantly different between concentric and

eccentric contractions. This phenomenon may be explained as a result of the training protocol. Due to the extensive muscle damage seen with eccentric contractions (Gibala et al., 1995, Gibala et al., 2000), it is likely that the frequent eccentric contractions did not allow the subjects to recover adequately between training sessions, thus strength gains did not increase as much as they may have with longer rest periods. Daily records of the peak torque attained for each training session reveal that a decrease in maximum torque generation was seen after the first week of training, and had only returned to pre testing values within the final week of training.

According to the present data, time under tension is not the integral factor in resistance training that was once hypothesized. Each individual contraction for the fast arm only lasted approximately 0.43 s, whereas the slow contractions lasted 4.5 s in duration. Each slow trained arm was under tension for a total of 3,510 s as opposed to 334 s for the fast trained arm. Hence, slow trained arms were under tension for 10.5 fold the duration of the fast trained arms, yet the fast training increased both muscle strength and hypertrophy to a greater degree than that of slow training.

The training protocol did not significantly alter the percent distribution of type I or IIa fibres found in biopsy samples according to ATPase histochemical staining; however, a significant difference was seen after training, in both arms, for a reduction in the percentage of type IIx fibres. Likewise, there was a significant decrease in the percent area occupied by type IIx fibres after training. An increase was observed in the percent area of IIa fibres after fast training, most likely attributed to the preferential type II fibre hypertrophy observed. In concordance with the distribution changes seen with histochemistry, MHC gel electrophoresis also showed a significant decrease in the percentage of type IIx isoform expression, with a trend ($P=0.08$) toward a type IIa increase.

Muscle fibre type transitions that occur with resistance training have usually demonstrated a shift from IIx to IIa fibres (Andersen et al., 1996, Williamson et al., 2000; Williamson et al., 2001). However, a recent observation of fibre type distribution after fast eccentric training showed an increase in the percentage of type IIx fibres and a decrease in type I fibres found within muscle samples (Paddon-Jones et al., 2001). Upon completion of the present 8-week training study, no such changes were observed. In fact, a decrease was seen in type IIx fibres after both fast and slow eccentric training according to both ATPase histochemical, and MHC gel electrophoretic analysis. Although the hypothesized concomitant increase in IIa fibre distribution was not seen, we did observe a strong trend for an increase in the MHC content of the IIa isoform. Moreover, a significant difference in the total area occupied by type IIa fibres was observed in the fast arm only. A shift from IIx to IIa fibres was to be expected according to Adams et al. (1993), who hypothesized that the type IIx isoform is the 'default' gene, which is expressed frequently in untrained subjects. However, when a training protocol is implemented, the MHC isoforms shift towards an increase in type IIa fibres that are generally thought to have a greater oxidative capacity, which confers upon them a greater resistance to fatigue.

Further research is warranted to more fully delineate the mechanisms which act to hypertrophy skeletal muscle. More specifically, whether or not eccentric contractions increase muscle protein synthesis to a greater degree than that of concentric contractions needs to be determined. Additionally, understanding the role of the muscle satellite cell in muscle hypertrophy and determine if more satellite cell activation is seen with greater ultrastructural muscle damage would be of great benefit to more completely understand muscle hypertrophy.

Conclusions

The results of the present study indicate that greater muscle hypertrophy is seen after 8 weeks of fast (3.66 rad/s) eccentric training when compared to slow (0.35 rad/s) eccentric training. Although it has not been confirmed, the likely mechanism for the greater hypertrophic response was due to greater ultrastructural muscle damage which was seen after fast eccentric contractions to a greater degree than slow eccentric contractions. Greater strength gains were also observed in the fast trained arms, most likely due to the increased number of contractile proteins associated with muscle hypertrophy. Muscle fibre type transitions were demonstrated to be in the IIx to IIa direction with a significant decrease in the percentage of IIx isoforms determined both histochemically and electrophoretically. The results indicate that time under tension is not an important factor in resistance training whereas the degree of muscle damage may be more consequential.

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APPENDIX 1

CONSENT FORM

SUBJECT SCREENING QUESTIONNAIRE

EXERCISE METABOLISM RESEARCH GROUP
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

INFORMATION & CONSENT TO PARTICIPATE IN RESEARCH

EFFECT OF HIGH AND LOW VELOCITY ECCENTRIC RESISTANCE TRAINING ON MUSCLE STRENGTH AND HYPERTROPHY

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.

INVESTIGATOR:	**DEPARTMENT:**	**CONTACT:**
Dr. Stuart Phillips	Kinesiology, IWC AB116	x27037 or x24465
Dr. Mark Tarnopolsky	Medicine, MUMC 4U5	x24465 or x76367
Tim Shepstone, B.A.	Kinesiology, IWC A103	x27037

PURPOSE:
Resistance exercise (weightlifting) is known to add new muscle, increasing strength and size of the trained muscle. It has also been confirmed that the use of eccentric (lengthening) contractions promotes this addition of muscle more then that of concentric (shortening) contractions. The mechanism for the increased response to the eccentric loads is thought to be due to the greater muscle damage that occurs with eccentric training. With this in mind, it is our hypothesis that eccentric contractions at a higher velocity will produce even greater gains in muscle mass and strength than that at slower speeds due to an even higher incidence of muscle damage. To determine if greater strength and size gains can be achieved and to understand the potential mechanism at higher eccentric contraction speeds we will analyze blood and muscle biopsy samples after an 8 week training protocol implementing both fast and slow eccentric contraction speeds.

DESCRIPTION OF TESTING PROCEDURES:

Prior to the commencement of the study you will be required to complete a routine medical screening including a health questionnaire. So long as you fulfill all criteria for entrance into the study you will then undergo the following: one (1) baseline muscle biopsy taken from each arm; baseline measurements of muscle electrical activity using electromyography during the bicep curl; baseline computerized Tomography (CT) scans from each arm; and you will have a blood sample taken from a vein in your arm (see "medical procedures" for description). 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 arm 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 arm 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 an arm randomly assigned to be trained using eccentric contractions at a high speed, while the other is trained in a similar fashion at a slow speed. You will then participate in an 8-week resistance training program, using the speeds that have been designated for each individual arm. There will be 3 training sessions per week, which will likely take under 30 minutes to perform. You will receive an honorarium for participation (see below). This weightlifting program will involve only eccentric (negative) bicep curls on the Biodex apparatus. The amount of training will start with only one set of each exercise, 10 repetitions at an intensity that is as high as you can sustain for each repetition. Each consecutive week you will perform an additional set on the training days until you reach a maximum of four sets, which will continue for the remainder of the eight weeks. In the fourth week of training, and again after completion of the 8 week resistance training program, you will report to the Exercise Metabolism Research Lab in the Ivor Wynne Centre where the same procedures that were completed on the baseline test day will be repeated, including biopsies, blood samples, CT scans, and electrical myography testing. **Hence, the total number of biopsies is four (4)** – each arm will have two biopsies each.

POTENTIAL RISKS AND DISCOMFORTS:

The potential risks of resistance training are minimal for a healthy adult. There is a potential chance of musculoskeletal injuries that include sprains and strains. This risk is very small and impossible to estimate when performing a well structured exercise program under the proper supervision. Since we are performing only one exercise the risks are minimized even further. There will be muscle damage of the biceps that will be present in the form of soreness and tenderness. The muscle soreness will be apparent 1-2 days following the training sessions, however, this response is natural and expected. Moreover, as your training progresses this response will decrease in severity and eventually become unnoticeable. 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 $450 upon the completion of the study to compensate you for your time commitment (total of ~35h). We will attempt to have the cheques ready for you immediately after completion of your testing. However, there may be a small delay (up to two weeks) before you get your money.

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 investigator has 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 & WITHDRAW:
You can choose whether to participate in this study or not. 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 that warrant doing so.

RIGHTS OF RESEARCH PARTICIPANTS:
You will receive a signed 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:

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INFORMATION:
You will be able to contact Dr. Phillips at 525-9140 (x24465 or x27037) and/or Tim Shepstone (x27037) and/or Dr. Tarnopolsky at 521-2100 (X75226) or 24hr. pager 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 SIGNED 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 PARTICIPANT DATE

WITNESS

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

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 medication?

   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

APPENDIX 2

MEDICAL PROCEDURES

METABOLISM RESEARCH GROUP
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

DESCRIPTION OF MEDICAL PROCEDURES

The study in which you are invited to participate involves four procedures which require medical involvement: muscle biopsy sampling, venous blood sampling, computerized tomography (CT) scans, and electromyography (EMG). 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 that might preclude your participation as a subject.

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 upper arm muscle (bicep brachii) 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 arm. There may be a small amount of bleeding from the incision, but this is minimal. The incision will be covered with sterile gauze. When the biopsy is taken a biopsy needle will be inserted into your arm through the incision. A piece of your muscle will then quickly be pulled into the needle and cut off. The piece of muscle removed is very small (~50-100 mg; about the size of the eraser on the end of a pencil). During the time that the sample is being taken (~4-5 sec), you may feel the sensation of deep pressure in your arm and on some occasions this is moderately painful. However, the discomfort very quickly passes and you are quite capable of performing exercise.

Following the biopsy, the incision 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; however, you are encouraged to stretch your muscle occasionally to prevent it from being stiff the following day. Once the anesthetic wears off, your arm may feel tight and often there is the sensation of a deep bruise. Should the need arise, you should not take any aspirin-based medicine (aspirin, ibuprofen, naprosyn) for 24 hours following the experiment as this can promote bleeding in the muscle. However, other pain medication such as acetaminophen (i.e. Tylenol®) is an acceptable alternative. It is also beneficial to keep your arm elevated, and the periodic application of an ice pack will help to reduce any swelling and residual soreness. The following day your arm will probably feel somewhat tight and may be uncomfortable when you lift objects. The tightness in the muscle usually disappears within 1-2 days, and subjects routinely begin exercising normally within 1-2 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 discolouration 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 3,500 have experienced a local skin infection; 1 in 1,100 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 4 months), and 1 in 60 have experienced mild bruising around the site of incision which lasted for ~4-5 days. There is a risk that a small vein may be cut during the procedure, however, careful examination of the bicep area, prior to the procedure, will minimize this possibility. In the event that the vein is cut you may experience some bleeding and bruising as a result. While there is also a theoretical risk of damage to small nerves of the bicep brachii, this has never been seen in biopsies performed at McMaster University in the IWC. Hence, the risk of damaging a small nerve is impossible to estimate.

Venous Blood Sampling

A small plastic catheter will be inserted into a forearm vein by a physician or a medically trained and certified member of the laboratory group. The catheter will be inserted with the assistance of a small needle, which will be subsequently removed. The discomfort of the 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 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.

Computerized Tomography (CT) Scans

The CT scan will be conducted on the upper arm across the bicep area. The arm will be inserted into a small cylinder which functions as the measurement area of the peripheral quantitative computer tomography (pQCT) densiometer. The arm must be motionless for approximately 4 minutes (the length of the scan). This procedure sends small amounts of x-ray beams through the arm and the attenuation with which the rays are passed through are recorded as the density of the arm. From this it can be determined what portion of the arm is bone, muscle or fat mass.

Potential Risks. There are no known risks or discomfort associated with this procedure. The use of the pQCT technique has been used extensively on individuals of all ages and involves safe and low doses of radiation (about half of the radiation of a typical chest x-ray and less than the amount of radiation received in a transatlantic flight).

Electromyography (EMG)

Small transducers called surface electrodes will be placed on the skin of the upper arm to sense the myoelectric activity within the particular muscle in question. This is a passive, non-invasive collection of data that records very small amounts of electrical current that the muscle makes through the firing of the nerves. This information is then stored in a computer to analyze the degree of neuromuscular function for each contraction. No current is sent into the muscle, but instead reads what is coming out of it.

Potential Risks. All precautions have been made to ensure that no current can be delivered to the muscle. Since nothing is going into the body, and only information is being received from the body, the potential risks are essentially zero.

APPENDIX 3

PHYSICAL CHARACTERISTICS

SUBJECT DATA – Training Study

Subject #	Age	Weight (kg)	Height (cm)
1	21	81	191
2	30	118	198
3	26	64	165
4	23	80	183
5	21	69	173
6*	22	87	180
7	21	71	161
8	24	91	181
9	23	88	175
10	20	77	177
11	24	82	182
12	23	86	178
13	31	78	176
Average	23.8	82.5	178.5
SD	3.4	13.3	9.6

* Indicates subject withdrew from study

SUBJECT DATA – Acute Study

Subject #	Age	Weight (kg)	Height (cm)
1	22	79	180
2	24	80	182
3	19	83	186
4	18	70	165
5	23	74	177
6	22	76	173
7	24	92	189
Average	21.7	79.1	178.9
SD	2.4	7.1	8.1

APPENDIX 4

STRENGTH TEST RAW DATA AND ANOVA TABLES

1 = TIME; 2 = CONDITION; 3 = CONTRACTION; 4 = VELOCITY

STRENGTH TEST DATA – Peak Torque (Nm)

PRE

	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast
Subject	210 Con		180 Con		120 Con		60 Con		20 Con		ISO		20 Ecc		60 Ecc		120 Ecc		180 Ecc		210 Ecc	
1	59.0	60.7	59.0	60.7	59.7	60.7	46.9	45.2	35.9	39.9	44.1	54.0	50.0	60.7	68.9	63.9	70.2	62.1	60.7	65.6	72.0	75.1
2	70.6	81.2	70.6	81.2	64.5	76.6	63.9	68.5	61.0	67.4	78.0	87.2	83.0	91.1	86.8	93.2	99.9	98.4	101.1	83.3	119.0	127.2
3	38.5	30.0	38.5	30.0	37.4	30.6	32.4	35.4	25.4	34.8	26.8	49.1	35.9	47.3	34.2	54.4	46.2	51.9	46.2	55.5	55.0	61.7
4	53.3	52.2	53.3	52.2	50.4	52.9	44.5	41.6	38.8	41.2	47.6	44.7	54.4	56.4	59.0	55.7	62.1	61.0	69.2	58.2	74.0	77.0
5	49.8	54.4	49.8	54.4	49.8	53.3	43.0	51.9	41.0	52.2	55.1	68.9	61.4	73.8	66.0	70.2	75.1	72.7	77.0	81.2	80.0	81.9
7	48.0	38.1	48.0	38.1	39.9	37.0	34.6	31.7	30.6	27.8	47.6	41.0	60.7	49.1	54.6	57.2	57.5	53.3	59.3	55.7	61.0	52.6
8	68.5	54.0	68.5	54.0	59.3	56.1	66.3	60.7	59.4	51.9	98.2	77.0	81.9	87.9	86.1	95.7	80.1	95.7	81.2	105.2	101.0	117.2
9	57.9	62.5	57.9	62.5	56.8	56.8	61.4	56.4	44.1	44.5	49.1	74.9	71.3	69.6	76.6	81.2	75.1	78.4	84.3	97.8	102.0	108.1
10	56.1	49.1	56.1	49.1	49.8	43.4	53.7	46.9	47.3	49.1	54.0	74.4	62.8	59.9	77.0	75.9	71.6	86.1	87.6	81.2	95.0	91.1
11	48.0	52.9	56.1	56.4	50.9	58.6	43.8	49.4	41.2	45.6	73.8	53.3	81.5	62.1	74.9	78.0	77.0	80.5	74.9	83.7	77.0	76.6
12	63.6	66.7	62.1	62.8	68.1	64.5	56.1	59.7	47.6	48.3	60.7	85.0	67.8	85.8	83.0	85.8	83.3	96.4	87.6	92.2	98.0	101.3
13	66.7	50.0	38.8	54.6	37.4	50.0	45.6	33.9	41.6	35.9	52.6	57.9	69.2	58.6	65.4	50.0	74.4	69.2	71.6	65.0	66.0	67.8
Mean	56.7	54.3	54.9	54.7	52.0	53.4	49.4	48.4	42.8	44.9	57.3	64.0	65.0	66.9	69.4	71.8	72.7	75.5	75.1	77.1	83.3	86.5
StDev	9.7	13.1	10.1	12.8	10.2	12.3	11.0	11.6	10.3	10.2	18.5	15.9	14.0	14.9	15.1	15.6	13.5	16.5	14.9	16.8	19.4	22.9

POST

	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast
Subject	210 Con		180 Con		120 Con		60 Con		20 Con		ISO		20 Ecc		60 Ecc		120 Ecc		180 Ecc		210 Ecc	
1	67.8	67.1	68.1	69.8	72.0	74.4	55.5	54.6	48.0	56.4	41.6	51.1	50.9	62.8	64.5	69.8	77.3	85.0	74.4	83.3	80.1	86.8
2	94.6	93.2	88.7	85.4	76.2	79.1	89.0	76.2	82.3	123	103	110	128	116	122	136	141	133	146.6	159.3	158.2	162.4
3	45.2	43.8	49.8	39.9	48.3	43.8	37.7	43.8	33.1	37.7	25.0	56.8	42.7	41.2	56.1	51.9	60.3	50.4	52.9	58.2	61.7	69.8
4	81.9	80.8	88.3	73.1	85.0	79.1	59.7	64.5	63.9	57.5	68.9	74.2	75.9	89.4	80.8	95.3	104	121	106.6	117.3	133.4	134.2
5	46.9	68.1	43.8	66.7	41.0	68.5	41.0	54.4	38.0	47.6	59.0	60.3	55.5	60.3	59.0	68.9	54.4	77.7	59.9	76.6	61.0	80.1
7	50.4	54.4	42.3	60.3	32.4	56.8	42.3	42.0	41.6	37.4	62.8	55.2	55.5	57.2	67.4	53.3	70.9	61.4	71.3	70.2	84.8	67.1
8	71.6	80.5	72.7	75.9	58.2	75.1	61.0	72.4	79.6	70.0	82.3	84.8	69.8	75.5	77.0	83.2	79.1	80.5	84.3	93.2	88.7	100.2
9	65.6	70.9	67.8	68.9	65.6	72.0	61.4	59.7	50.9	48.3	53.7	61.7	57.0	63.2	63.2	72.7	77.7	84.1	77.3	88.3	83.7	92.9
10	72.0	65.0	67.4	60.3	63.9	58.6	57.9	50.4	49.4	50.4	56.4	57.9	72.4	61.4	71.6	67.8	75.5	78.8	83.7	83.7	93.6	91.4
11	62.8	63.6	63.2	51.1	55.7	50.0	43.0	48.7	47.3	75.2	68.5	63.9	68.9	55.7	74.9	67.8	79.7	79.5	84.3	82.3	84.3	89.6
12	77.0	83.0	81.5	87.9	68.9	79.7	69.2	72.4	48.0	73.1	72.7	99.5	79.7	90.7	81.2	94.9	94.6	98.2	96.7	122.9	102.1	127.9
13	63.2	52.2	50.0	54.0	57.2	47.3	42.3	41.6	33.9	37.0	52.6	45.2	47.3	55.5	57.5	66.7	58.2	63.2	61.7	70.6	62.5	65.4
Mean	66.6	68.6	65.3	66.1	60.4	65.4	55.0	56.7	51.3	59.5	62.2	68.4	67.0	69.0	72.9	77.4	81.1	84.4	83.3	92.2	91.2	97.3
StDev	14.5	14.3	16.2	13.9	14.9	13.3	14.9	12.3	16.1	24.1	19.7	19.9	22.5	20.4	17.7	23.0	23.7	23.6	25.1	28.1	29	29.8

Time x Training Condition x Contraction Type x Test Velocity – 4-Way ANOVA

1-TIME, 2-CONDITION, 3-CONTRACTION, 4-VELOCITY

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	9302.48242	11	1431.7251	6.497395	**0.027043**
2	1	895.713501	11	182.883972	4.897715	**0.048954**
3	1	56136.3398	11	514.905212	109.0227	**4.79E-07**
4	4	4456.99268	44	74.1236115	60.12919	**2.87E-17**
12	1	278.008514	11	56.0921555	4.956282	**0.047838**
13	1	283.82251	11	409.853882	0.692497	0.423015
23	1	112.81102	11	99.2626572	1.13649	0.309235
14	4	140.353714	44	39.4372864	3.558909	**0.013381**
24	4	10.4776354	44	24.5924072	0.426052	0.788993
34	4	371.962769	44	58.9166222	6.313376	**0.000418**
123	1	7.5250206	11	91.8185654	0.081955	0.779985
124	4	3.89055204	44	27.2455521	0.142796	0.965233
134	4	88.4457016	44	38.8524017	2.276454	0.07613
234	4	74.7644043	44	32.0510445	2.332667	0.07049
1234	4	28.1819477	44	35.6800385	0.789852	0.538063

Test Velocity – Post Hoc

		{1} 58.29792	{2} 62.61979	{3} 68.10000	{4} 71.06771	{5} 75.55104
20°/s	{1}		**0.0097574**	**0.0001299**	**0.00012994**	**0.00013**
60°/s	{2}	**0.0097574**		**0.0007343**	**0.00013012**	**0.00013**
120°/s	{3}	**0.000129938**	**0.00073433**		0.13781244	**0.000132**
180°/s	{4}	**0.000129938**	**0.00013012**	0.1378124		**0.006812**
210°/s	5}	**0.000129938**	**0.00012994**	**0.000132**	**0.00681239**	

Time x Training Condition Interaction – Post Hoc

		{1} 63.33000	{2} 62.12000	{3} 73.65667	{4} 69.40250
Pre Fast	{1}		0.60962713	**0.0001954**	**0.00046337**
Pre Slow	{2}	0.609627128		**0.0001953**	**0.00023413**
Post Fast	{3}	**0.000195444**	**0.00019532**		**0.00511599**
Post Slow	{4}	**0.000463367**	**0.00023413**	**0.005116**	

DAILY STRENGTH DATA – Peak Torque (Nm)

Sub#	Arm	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	210	96	87.2	75.1	74.4	59.9	68.5	61	80.8	78.4	91.4	79.7	85.8	92.9	95.7	99.3	94.6	96.7	98.9	97.8	94	94.9	91.1
	20	71.3	69.8	48	50.9	41.6	35.7	39.5	37.7	47.6	59.9	55.1	61	56.4	62.8	65	65	70.2	71	71.3	73.8	69.2	67.4
2	210	127	133	133	115	110	111	113	123	125	134	109	112	126	125	123	113	115	116	117	125	161	188
	20	92.5	95.7	79.1	68.1	65	58.6	62.5	83.7	72	82.3	86.1	92.5	80.5	84.8	96.7	111	116	93	105	120	149	158
3	210	61.7	61.7	61.7	78.4	60.7	61	56.1	70.6	69.8	65.6	70	71.3	63.9	66	78.8	79.5	82.6	83	89.4	76.6	72.4	90.4
	20	37	43.4	37.7	38.5	35	32.4	35.3	27.1	39.9	41	32.8	39.5	29.6	38.5	24.7	24.7	34.2	36	41.2	41.2	42	37
4	210	92.2	77	66	84.1	83.7	81.5	78.8	67.1	93.6	92	96	85	82.3	87.9	94.9	104	106	94	92.9	100	133	132
	20	76.6	56.8	47.6	48.7	43.8	41.2	52.9	75.1	86.5	76.3	59	81.9	64.5	92.9	98.4	110	78.8	84	86.5	114	99.3	112
5	210	81.9	86.8	86.1	76.2	82.3	78.4	70.6	72	81.9	83	87.2	85	84.3	86.5	82.3	82.3	71.3	81	79.7	91.8	85.8	82.3
	20	70.9	73	75.1	62.5	55.5	52.2	48.7	52.9	46.5	60	65.6	72.7	66	59.9	60.7	60.7	58.6	60	61.7	54	60.3	60.7
7	210	52.6	64.3	58.2	73.1	54.6	51.5	59	63.6	61	63	63.9	73.1	74.2	62.1	65	72.7	76.2	65	61	65	73.8	73.8
	20	49.1	49.1	42.3	44.5	33.1	37.4	39.2	52.6	39.9	49	50.4	56.4	51.9	56.4	52.2	52.2	55.1	54.4	54.6	49	41.2	69.8
8	210	117	114	105	104	79.7	89.6	106	113	99.9	114	107	119	98.2	88.7	107	94.6	99.9	109	97.8	99.9	108	107
	20	83.3	82.3	67.4	74.4	65.4	69.8	61.4	68.1	77.7	83.7	81.9	84.3	74.4	83	82.6	83	85	86.8	84.8	85.8	94.9	83
9	210	108	106	101	92.2	79.7	71.3	74.2	75.9	86.1	96.7	85	103	91.4	90	98.2	89	94.2	96.7	109	114	107	107
	20	93.2	87.9	65	61.7	48.7	50.4	48.7	46.9	59	63	68.5	68.1	54.4	59.7	61	57.2	64.3	72.4	50.4	70.6	73.1	70.2
10	210	91.1	102	73.4	93.2	72.4	92.2	91.1	93.2	87.2	94.6	104	94.2	103	92.5	90.7	85	95	92.9	95.7	94	92	91.4
	20	63.6	62.5	55.7	60.7	60.7	54.4	67.8	79.1	69.8	68.9	75.1	70.6	68.5	67.1	67.1	62.1	59.7	65	68.1	79.7	70	68.1
11	210	84.1	81.5	87.6	83.7	83	85.4	93.2	86.5	83.3	87.6	85	78	84.1	87.9	87.2	84.1	86.8	81.5	92.5	97.8	85.4	93.2
	20	67.8	72	67.1	66.7	65	69.2	71.3	72	75.1	71.6	70.2	73.8	76.6	79.1	79.5	76.6	78.4	73.8	79.7	83	78.4	81.2
12	210	105	106	108	112	99.5	94.9	101	113	118	105	106	105	96.4	109	122	119	102	101	91.8	111	121	124
	20	78.8	83.3	84.1	87.9	86.8	80.8	85.8	81.5	85.4	90.7	88.3	85	88.3	98.2	92.2	94.2	70.6	87.6	83.7	92.9	85.4	85.4
13	210	68.9	84.3	88.7	76.2	81.2	73.4	83.3	74.2	89.6	74.2	61.7	67.8	84.1	59.3	58.2	68.9	55.5	80.1	78.8	78.8	67.8	61.7
	20	48.3	64.3	58.6	53.3	43.4	43.8	41.2	33.5	48	49.1	39.5	45.6	29.6	42.7	42.7	44.7	37.4	37.4	43	45.8	35.7	41.6
Avg	210	90.5	92	87.1	88.5	78.9	79.9	82.4	86.1	89.5	91.7	87.8	90	90	87.6	92.2	90.6	90.1	91.6	92	95.6	100	104
SD	20	22.3	21	22.1	14.8	15.8	16.2	19.1	20.2	18.2	20	16.8	16.8	15.5	18.9	19.9	15.3	16.5	14.1	14.5	16.7	27.6	33.2
Avg	210	69.4	70	60.6	59.8	53.7	52.2	54.5	59.2	62.3	66.3	64.4	69.3	61.7	68.8	68.6	70.1	67.3	68.5	69.1	75.8	74.9	77.8
SD	20	17.5	15.8	14.9	13.7	15.6	15.2	15.4	19.9	17.5	15.4	17.7	16.2	18.5	19	22.5	26	21.7	18.8	19.6	25.6	31.2	32

Daily Strength ANOVA Table

1-TIME, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	21	908.465	231	154.6406	5.874686	**9.43E-13**
2	1	77665.48	11	584.8676	132.7916	**1.76E-07**
12	21	52.88051	231	56.24645	0.940157	0.539541

	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}	{19}	{20}	{21}	{22}
	79.93	81.03	73.85	74.18	66.27	66.02	68.44	72.65	75.90	79.01	76.11	79.62	75.88	78.18	80.38	80.33	78.70	80.03	80.56	85.72	87.57	90.68
{1}		1.000	0.988	0.994	**0.024**	**0.019**	0.161	0.919	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.993	0.876	0.266
{2}	1.000		0.929	0.955	**0.007**	**0.006**	0.066	0.757	0.999	1.000	0.999	1.000	0.999	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.972	0.482
{3}	0.988	0.929		1.000	0.884	0.849	0.997	1.000	1.000	0.999	1.000	0.994	1.000	1.000	0.972	0.975	0.999	0.985	0.964	0.121	**0.023**	**0.001**
{4}	0.994	0.955	1.000		0.838	0.796	0.994	1.000	1.000	0.999	1.000	0.997	1.000	1.000	0.984	0.986	1.000	0.992	0.979	0.155	**0.031**	**0.001**
{5}	**0.024**	**0.007**	0.884	0.838		1.000	1.000	0.979	0.488	0.058	0.442	**0.033**	0.493	0.116	**0.015**	**0.016**	0.076	**0.022**	**0.012**	**0.000**	**0.000**	**0.000**
{6}	**0.019**	**0.006**	0.849	0.796	1.000		1.000	0.967	0.434	**0.046**	0.390	**0.026**	0.438	0.095	**0.012**	**0.012**	0.061	**0.017**	**0.010**	**0.000**	**0.000**	**0.000**
{7}	0.161	0.066	0.997	0.994	1.000	1.000		1.000	0.900	0.297	0.873	0.201	0.902	0.463	0.114	0.118	0.356	0.150	0.099	**0.000**	**0.000**	**0.000**
{8}	0.919	0.757	1.000	1.000	0.979	0.967	1.000		1.000	0.979	1.000	0.947	1.000	0.996	0.864	0.871	0.989	0.909	0.838	**0.043**	**0.006**	**0.000**
{9}	1.000	0.999	1.000	1.000	0.488	0.434	0.900	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.446	0.140	**0.007**
{10}	1.000	1.000	0.999	0.999	0.058	**0.046**	0.297	0.979	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.964	0.720	0.141
{11}	1.000	0.999	1.000	1.000	0.442	0.390	0.873	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.492	0.164	**0.009**
{12}	1.000	1.000	0.994	0.997	**0.033**	**0.026**	0.201	0.947	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.987	0.831	0.217
{13}	1.000	0.999	1.000	1.000	0.493	0.438	0.902	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	0.441	0.138	**0.007**
{14}	1.000	1.000	1.000	1.000	0.116	**0.095**	0.463	0.996	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	0.890	0.541	0.072
{15}	1.000	1.000	0.972	0.984	**0.015**	**0.012**	0.114	0.864	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	0.998	0.928	0.348
{16}	1.000	1.000	0.975	0.986	**0.016**	**0.012**	0.118	0.871	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	0.997	0.923	0.338
{17}	1.000	1.000	0.999	1.000	0.076	0.061	0.356	0.989	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	0.942	0.654	0.110
{18}	1.000	1.000	0.985	0.992	**0.022**	**0.017**	0.150	0.909	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	0.995	0.889	0.282
{19}	1.000	1.000	0.964	0.979	**0.012**	**0.010**	0.099	0.838	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		0.999	0.943	0.383
{20}	0.993	1.000	0.121	0.155	**0.000**	**0.000**	**0.000**	**0.043**	0.446	0.964	0.492	0.987	0.441	0.890	0.998	0.997	0.942	0.995	0.999		1.000	0.999
{21}	0.876	0.972	**0.023**	**0.031**	**0.000**	**0.000**	**0.000**	**0.006**	0.140	0.720	0.164	0.831	0.138	0.541	0.928	0.923	0.654	0.889	0.943	1.000		1.000
{22}	0.266	0.482	**0.001**	**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	**0.007**	0.141	**0.009**	0.217	**0.007**	0.072	0.348	0.338	0.110	0.282	0.383	0.999	1.000	

APPENDIX 5

CT SCAN RAW DATA AND ANOVA TABLES

1 = TIME; 2 = CONDITION

WHOLE MUSCLE CSA DATA – Mean Area (mm2)

	PRE		POST	
Subject #	**Fast Arm**	**Slow Arm**	**Fast arm**	**Slow Arm**
1	4114.3	4247.1	4457.6	4592.5
2	4945.9	4822.1	5204.8	5057.1
3	3599.2	3190.4	4198.7	3852.1
4	3843.6	4264.3	3957.3	4250.4
5	4183	3935.7	4233.1	4011
7	4603	4761.4	4976.9	5097.4
8	5798.4	5542.5	6032	5759.2
9	5640.5	5612.3	6706.1	6092.6
10	5226.3	5111.2	5680.6	5395.8
11	5104.2	5436.2	5168.5	5368.6
12	6333.3	6332.8	6482.2	6364.8
13	3928.2	3958.7	4064.4	4022.9
Mean	4776.66	4767.89	5096.85	4988.70
St. Dev.	871.24	888.82	957.33	848.26

Mean Whole Muscle CSA

1-TRAINING, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	878043	11	57676.988	15.22345	**0.002469**
2	1	41008.5195	11	56999.477	0.719454	0.414399
12	1	29631.1406	11	7095.0024	4.17634	0.065695

APPENDIX 6

MUSCLE FIBRE SIZE RAW DATA AND ANOVA TABLES

1 = TIME; 2 = CONDITION

FIBRE SIZE DATA – Mean Area (μm2)

Pre Training				Post Training			
Subject	**Type I**	**Type IIx**	**Type IIa**	**Subject**	**Type I**	**Type IIx**	**Type IIa**
Fast Training							
S1-L	3291.8	2998.9	4295.7	S1-L	3635.0	3962.2	5330.5
S2-R	3086.9	3143.7	4232.7	S2-R	3813.3	4460.6	5346.6
S3-R	2758.1	2371.2	3133.8	S3-R	3394.4	3696.9	4398.8
S4-L	4029.9	4180.1	4975.9	S4-L	5186.7	5515.9	6227.1
S5-R	4193.3	4935.9	6256.7	S5-R	4089.2	6666.7	6332.9
S7-R	3430.9	3547.5	4777.1	S7-R	3898.2	3826.5	5363.1
S8-R	4828.8		7648.5	S8-R	4922.6	4682.1	8758
S9-L	4057.7	4923.4	7020.5	S9-L	3949.9	5891.2	7241.9
S10-L	3231.9	4854.2	5574.2	S10-L	4528.7	6093.9	7075.9
S11-L	4083.3		5087.8	S11-L	4222.0		6177.9
S12-L	3744.6	3612.9	5418.9	S12-L	4392.2		8263.9
S13-R	2252.4	2666.4	3349.8	S13-R	2946.8	4214.4	4404.1
Mean	3582.5	3723.4	5147.6	Mean	4081.6	4901.0	6243.4
StDev	710.6	957.3	1357.4	StDev	627.2	1059.3	1391.7
Slow Training							
S1-R	3613.6	3522.2	4440.1	S1-R	3270.2	3464.9	5129.8
S2-L	3336.9	4016.6	5158.1	S2-L	3516.3	4577.3	5716.5
S3-L	2545.5	2661.7	3727.8	S3-L	3108.6	3418.0	4027.3
S4-R	3597.0	3200.1	4611.1	S4-R	3287.2	3679.6	4815.9
S5-L	4755.2	7343.0	7632.1	S5-L	5582.0	7956.4	8755.8
S7-L	4130.4	4543.0	6103.5	S7-L	4271.9	4166.9	5817.6
S8-L	*	*	*	S8-L	4510.3	6286.7	6227.9
S9-R	6277.6	7015.3	10432.9	S9-R	6195.1		11081.5
S10-R	4908.3		7432.1	S10-R	4762.3	5860.5	7534.9
S11-R	4101.8		4962.8	S11-R	4022.6	4171.5	5359.5
S12-R	3635.1	5023.3	5793	S12-R	3904.4	4710.3	6421.9
S13-L	1912.9	2450.9	2831.6	S13-L	1915.9	3417.2	3660.1
Mean	3999.4	4694.4	5871.1	Mean	4028.9	4700.8	6212.4
StDev	1181.5	1882.1	2074.3	StDev	1154.0	1445.8	2078.3

* Indicates no measurable cross-sectional fibres were found. Subject was omitted from statistical analysis.

Mean Fibre Size – Type I Fibres

1-TRAINING, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	1087635.4	10	55250.039	19.68569	**0.001261**
2	1	446745.66	10	878295.63	0.508651	0.492031
12	1	539773.38	10	112286.66	4.807101	0.05311

Mean Fibre Size – Type IIa Fibres

1-TRAINING, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	6751145.5	10	150447.13	44.87387	**5.37E-05**
2	1	2830759.3	10	1843968.1	1.535146	0.243635
12	1	1064537.5	10	190902.16	5.576352	**0.039856**

		{1} 4920.259	{2} 5738.636	{3} 6014.764	{4} 6210.964
Pre Fast	{1}		**0.006309**	**0.00091106**	**0.0003694**
Pre Slow	{2}	**0.00630897**		0.48211074	0.1139634
Post Fast	{3}	**0.00091106**	0.4821107		0.7238708
Post Slow	{4}	**0.00036943**	0.1139634	0.72387075	

Mean Fibre Size – Type IIx Fibres

1-TRAINING, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	4675937	6	212454.81	22.00909	**0.003356**
2	1	177746.95	6	1028216.6	0.172869	0.692035
12	1	1102747	6	11502.278	95.87205	**6.53E-05**

		{1} 3406.243	{2} 3962.500	{3} 4620.457	{4} 4382.900
Pre Fast	{1}		**0.0004712**	**0.00024492**	**0.000246**
Pre Slow	{2}	**0.00047117**		**0.00030714**	**0.0015283**
Post Fast	{3}	**0.00024492**	**0.0003071**		**0.0234703**
Post Slow	{4}	**0.00024599**	**0.0015283**	**0.02347028**	

Δ FIBRE SIZE DATA – Mean Area (μm2)

Fast Training				Slow Training			
Subject	**Type I**	**Type IIx**	**Type IIa**	**Subject**	**Type I**	**Type IIx**	**Type IIa**
Fast Training							
S1-L	343.2	963.3	1034.8	S1-R	-343.4	-57.3	689.7
S2-R	726.4	1316.9	1113.9	S2-L	179.4	560.7	558.4
S3-R	636.3	1325.7	1265.1	S3-L	563.1	756.3	299.5
S4-L	1156.8	1335.8	1251.2	S4-R	-309.8	479.5	204.9
S5-R	-104.1	1730.8	76.2	S5-L	826.8	613.4	1123.7
S7-R	467.3	279.0	586.0	S7-L	141.5	-376.1	-285.9
S8-R	93.8		1109.5	S8-L	*	*	*
S9-L	-107.8	967.8	221.4	S9-R	-82.5		648.6
S10-L	1296.8	1239.7	1501.7	S10-R	-146.0		102.8
S11-L	138.7		1090.1	S11-R	-79.2		396.7
S12-L	647.6		2845.0	S12-R	269.3	-313.0	628.9
S13-R	694.4	1548.0	1054.3	S13-L	3.0	966.3	828.5
Mean	499.1	1189.7	1095.8	Mean	92.9	328.7	472.3
StDev	452.4	419.8	697.2	StDev	357.6	507.7	384.7

* Indicates no measurable cross-sectional fibres were found. Subject was omitted from statistical analysis.

Paired T-Tests

Pre-Post Difference of Fibre Size – Type I Fibres

	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
FDIFFI	535.9636	455.1936						
SDIFFI	92.92727	357.5923	11	443.0364	670.184	2.19251	10	0.0531

Pre-Post Difference of Fibre Size – Type IIa Fibres

	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
FDIFFIIA	1094.505	731.2405						
SDIFFIIA	472.3273	384.6894	11	622.1773	873.847	2.36143	10	**0.03985589**

Pre-Post Difference of Fibre Size – Type IIx Fibres

	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
FDIFFIIX	1214.214	474.9761						
SDIFFIIX	420.4	471.4996	7	793.8143	214.4973	9.791427	6	**6.53E-05**

APPENDIX 7

MUSCLE FIBRE TYPE RAW DATA AND ANOVA TABLES

1 = TIME; 2 = CONDITION

FIBRE TYPE DATA – Percent Distribution

Pre Training				Post Training			
Subject	**Type I**	**Type IIx**	**Type IIa**	**Subject**	**Type I**	**Type IIx**	**Type IIa**
Fast Training							
S1-L	34.4	15.4	50.2	S1-L	42.7	2.5	54.9
S2-R	78.7	4.6	16.7	S2-R	40.4	9.8	49.7
S3-R	39.7	9.6	50.7	S3-R	22.5	16.7	60.8
S4-L	39.3	18.0	42.7	S4-L	47.6	9.5	42.9
S5-R	46.2	7.0	46.8	S5-R	52.4	2.0	45.6
S7-R	34.0	24.4	41.7	S7-R	32.1	9.6	58.3
S8-R	43.3	0.0	56.7	S8-R	48.1	7.6	44.3
S9-L	44.2	4.9	50.9	S9-L	42.5	1.9	55.6
S10-L	54.1	0.4	45.5	S10-L	53.6	2.8	43.6
S11-L	28.9	0.0	71.2	S11-L	29.9	0.0	70.1
S12-L	77.0	4.4	45.1	S12-L	56.1	0.0	43.9
S13-R	43.6	22.1	34.3	S13-R	38.6	19.9	41.5
Mean	46.9	9.2	46.0	Mean	42.2	6.9	50.9
StDev	15.8	8.7	12.9	StDev	10.2	6.5	9.0
Slow Training							
S1-R	47.6	1.8	50.7	S1-R	50.5	15.8	33.7
S2-L	48.2	21.1	30.7	S2-L	46.4	5.1	48.6
S3-L	24.1	36.5	39.4	S3-L	48.0	13.0	39.0
S4-R	56.9	3.8	39.2	S4-R	60.2	7.0	32.8
S5-L	34.5	9.4	56.1	S5-L	62.4	5.9	31.8
S7-L	51.2	22.3	26.5	S7-L	32.8	14.8	52.5
S8-L	37.0	10.9	52.2	S8-L	43.1	1.0	55.9
S9-R	51.8	0.9	47.4	S9-R	48.5	0.0	51.5
S10-R	62.9	0.0	37.1	S10-R	63.8	1.3	34.9
S11-R	28.3	0.0	71.7	S11-R	32.5	7.3	60.3
S12-R	62.1	1.0	36.9	S12-R	66.9	3.3	29.8
S13-L	38.5	27.0	34.5	S13-L	42.9	14.6	42.6
Mean	45.3	11.2	43.5	Mean	49.8	7.4	42.8
StDev	12.7	12.5	12.6	StDev	11.5	5.8	10.6

Percent Distribution – Type I Fibres

1-TRAINING, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	0.075208	11	88.78709	0.000847	0.977303
2	1	105.4354	11	60.32345	1.747834	0.212982
12	1	259.563	11	80.35117	3.230357	0.099761

Percent Distribution – Type IIa Fibres

1-TRAINING, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	55.27667	11	163.6106	0.337855	0.572787
2	1	332.169	11	73.8446	4.498217	0.05748
12	1	101.7627	11	53.48901	1.902498	0.195195

Percent Distribution – Type IIx Fibres

1-TRAINING, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	114.639	11	18.85406	6.080336	**0.031352**
2	1	19.40563	11	23.53313	0.824609	0.383299
12	1	6.149008	11	63.41219	0.096969	0.76132

FIBRE TYPE DATA – Percent Area

Pre Training				Post Training			
Subject	**Type I**	**Type IIx**	**Type IIa**	**Subject**	**Type I**	**Type IIx**	**Type IIa**
Fast Training							
S1-L	23.2	9.4	67.4	S1-L	22.8	1.4	75.8
S2-R	63.2	9.4	27.4	S2-R	28.4	8.1	63.5
S3-R	28.8	6.0	65.2	S3-R	15.6	12.5	71.9
S4-L	34.4	7.4	58.2	S4-L	35.2	7.4	57.4
S5-R	31.1	5.6	63.3	S5-R	33.9	2.2	63.9
S7-R	26.3	19.5	54.2	S7-R	21.4	6.3	72.3
S8-R	23.6	19.5	56.9	S8-R	28.2	4.2	67.6
S9-L	27.9	0.0	72.1	S9-L	19.5	1.3	79.2
S10-L	32.5	3.3	64.2	S10-L	35.5	2.5	62.0
S11-L	24.5	0.0	75.5	S11-L	22.5	0.0	77.5
S12-L	48.4	0.0	51.6	S12-L	40.4	0.0	59.6
S13-R	36.1	21.7	42.2	S13-R	29.9	22.1	48.0
Mean	33.3	8.5	58.2	Mean	27.8	5.7	66.6
StDev	11.7	7.8	13.3	StDev	7.5	6.4	9.3
Slow Training							
S1-R	33.7	1.2	65.1	S1-R	32.1	10.6	57.3
S2-L	36.5	19.2	44.3	S2-L	29.7	4.2	66.1
S3-L	16.4	25.9	57.7	S3-L	33.2	9.9	56.9
S4-R	44.3	2.7	53.0	S4-R	39.5	5.1	55.4
S5-L	17.8	7.5	74.7	S5-L	37.6	5.1	57.3
S7-L	37.3	17.9	44.8	S7-L	24.2	10.6	65.2
S8-L	21.6	8.8	69.6	S8-L	31.2	1.1	67.7
S9-R	25.7	0.5	73.8	S9-R	25.9	0.0	74.1
S10-R	51.0	0.0	49.0	S10-R	43.5	1.1	55.4
S11-R	24.6	0.0	75.4	S11-R	27.7	5.6	66.7
S12-R	50.7	1.2	48.1	S12-R	55.6	3.5	40.9
S13-L	31.0	27.5	41.5	S13-L	28.5	17.3	54.2
Mean	32.6	9.4	58.1	Mean	34.1	6.2	59.8
StDev	11.9	10.5	13.0	StDev	8.9	5.0	8.7

Percent Area – Type I Fibres

1-TRAINING, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	49.2075	11	63.11568	0.77964	0.396138
2	1	90.75	11	51.88546	1.749045	0.212834
12	1	149.8133	11	42.93424	3.489367	0.088614

Percent Area – Type IIa Fibres

1-TRAINING, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	303.5102	11	101.7775	2.982096	0.112127
2	1	142.4852	11	54.29248	2.6244	0.133521
12	1	134.3352	11	22.66975	5.925746	**0.033157**

	{1} 58.18333	{2} 58.08333	{3} 66.55833	{4} 59.76667
Pre Fast {1}		0.999952	**0.005909**	0.846465
Pre Slow {2}	0.999952		**0.00545**	0.822081
Post Fast {3}	**0.005909**	**0.00545**		**0.022337**
Post Slow {4}	0.846465	0.822081	**0.022337**	

Percent Area – Type IIx Fibres

1-TRAINING, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	108.3002	11	23.79021	4.552302	0.056226
2	1	5.810208	11	14.97294	0.388047	0.546026
12	1	0.421875	11	28.41369	0.014848	0.905215

APPENDIX 8

MHC RAW DATA AND ANOVA TABLES

1 = TIME; 2 = CONDITION

MHC DATA – Percent Expression

Pre Training				Post Training			
Subject	**Type I**	**Type IIx**	**Type IIa**	**Subject**	**Type I**	**Type IIx**	**Type IIa**
Fast Training							
S1-L	38.1	3.3	58.6	S1-L	38.1	0.6	61.3
S2-R	54.3	6.4	39.3	S2-R	38.7	11.0	50.3
S3-R	30.2	14.6	55.2	S3-R	32.5	4.1	63.4
S4-L	40.9	16.0	43.1	S4-L	34.9	6.2	58.9
S5-R	30.1	6.9	63.0	S5-R	36.7	0.0	63.3
S7-R	29.5	17.2	53.3	S7-R	21.4	7.3	71.3
S8-R	34.7	12.5	52.8	S8-R	32	6.5	61.5
S9-L	35.1	1.1	63.8	S9-L	30.3	0.0	69.7
S10-L	32	6.9	61.1	S10-L	48.7	0.8	50.5
S11-L	33.6	0.0	66.4	S11-L	40.4	8.1	51.5
S12-L	52.4	0.0	47.6	S12-L	46.5	0.7	52.8
S13-R	34.6	11.7	53.7	S13-R	33	0.0	67.0
Mean	37.1	8.1	54.8	Mean	36.1	3.8	60.1
StDev	8.3	6.2	8.4	StDev	7.3	3.9	7.4
Slow Training							
S1-R	34.7	9.2	56.1	S1-R	33.1	8.8	58.1
S2-L	34.2	23.5	42.3	S2-L	27.9	9.6	62.5
S3-L	23.5	15.0	61.5	S3-L	34.4	11.4	54.2
S4-R	51.7	1.6	46.7	S4-R	44.1	1.4	54.5
S5-L	31.8	0.0	68.2	S5-L	46.8	0.0	53.2
S7-L	47.3	12.3	40.4	S7-L	21.5	13.5	65.0
S8-L	37.8	5.9	56.3	S8-L	30.5	3.1	66.4
S9-R	33.5	0.0	66.5	S9-R	25.7	0.0	74.3
S10-R	55.4	0.2	44.4	S10-R	37.8	0.0	62.2
S11-R	21	0.0	79.0	S11-R	33.3	0.0	66.7
S12-R	44.5	0.0	55.5	S12-R	43	0.9	56.1
S13-L	29.6	15.8	54.6	S13-L	31.8	6.9	61.3
Mean	37.1	7.0	56.0	Mean	34.2	4.6	61.2
StDev	10.7	8.1	11.6	StDev	7.6	5.1	6.3

MHC Percent Expression – Type I Fibres

1-TRAINING, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	46.8075	11	67.03204	0.698285	0.421141
2	1	11.80083	11	59.42265	0.198592	0.664504
12	1	10.83	11	40.38182	0.26819	0.614804

MHC Percent Expression – Type IIa Fibres

1-TRAINING, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	333.9075	11	91.24932	3.659288	0.082132
2	1	14.74083	11	38.10083	0.38689	0.546615
12	1	0.0075	11	35.24113	0.000213	0.988622

MHC Percent Expression – Type IIx Fibres

1-TRAINING, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	130.68	11	13.78091	9.482683	**0.010483**
2	1	0.163333	11	28.77242	0.005677	0.941294
12	1	11.4075	11	16.64205	0.685463	0.42531

APPENDIX 9

EVOKED TWITCH CHARACTERISTICS RAW DATA

AND ANOVA TABLES

1 = TIME; 2 = CONDITION

TWITCH PROTOCOL DATA – Peak Torque (Nm), *time* (s)

Fast Arm									Slow Arm							
Subj.	Day 0		Day 1		Day 2		Day 4		Subj.	Day 0		Day 1		Day 2		Day 4	
	Peak	*t to P*	Peak	*t to P*	Peak	*t to P*	Peak	*t to P*		Peak	*t to P*	Peak	*t to P*	Peak	*t to P*	Peak	*t to P*
Twitch 1																	
AS1	11.7	52.2	8.7	54.2	8.8	52.1	10.5	50.8	AS1	9.1	48.3	5.8	50.8	5.0	50.0	5.5	53.4
AS2	13.2	81.4	8.9	53.2	10.3	50.0	7.6	59.8	AS2	10.4	55.8	5.6	49.1	4.5	40.6	2.7	43.8
AS3	12.9	72.6	14.3	58.5	15.5	69.7	9.0	80.6	AS3	14.7	51.2	10.8	48.4	13.0	59.6	8.3	69.4
AS4	7.4	58.6	6.7	58.3	6.1	57.8	6.1	56.2	AS4	9.3	55.8	5.5	52.3	5.7	47.1	5.5	54.8
AS5	10.7	49.2	7.3	47.5	7.5	46.6	6.1	48.1	AS5	10.1	48.9	5.2	49.2	4.3	41.0	4.6	45.3
AS6	11.2	58.9	*	*	11.6	52.6	10.4	53.2	AS6	12.2	51.3	*	*	9.8	52.6	9.7	49.1
AS7	13.8	62.7	9.2	65.0	13.2	60.4	13.3	64.5	AS7	12.3	54.6	12.3	69.3	15.3	89.0	15.3	68.3
Mean	11.6	62.2	9.2	56.1	10.4	55.6	9.0	59.0	Mean	11.1	52.3	7.5	53.2	8.2	54.3	7.4	54.9
StDev	2.1	11.3	2.7	5.9	3.3	7.7	2.7	11.0	StDev	2.0	3.2	3.2	8.0	4.5	16.7	4.2	10.3
MVC		**Interp.**		**Interp.**		**Interp.**		**Interp.**			**Interp.**		**Interp.**		**Interp.**		**Interp.**
AS1	59.9	0.0	60.5	2.7	66.1	2.1	72.9	2.1	AS1	57.3	0.0	52.4	0.8	51.2	0.1	57.8	0.0
AS2	71.7	0.0	67.4	0.0	62.5	0.7	66.0	0.2	AS2	53.4	0.0	36.8	0.0	39.5	0.2	40.4	0.1
AS3	66.1	0.2	37.3	0.3	67.8	0.1	64.9	0.8	AS3	69.6	0.4	58.1	0.7	56.1	0.1	57.0	0.9
AS4	60.9	0.2	56.4	0.0	54.9	0.0	57.1	0.2	AS4	68.7	0.0	60.0	0.5	56.2	1.2	68.4	0.1
AS5	92.4	0.6	69.4	1.5	68.1	0.4	73.2	0.7	AS5	73.3	0.2	55.7	0.0	57.1	0.2	53.5	0.2
AS6	*	*	*	*	*	*	*	*	AS6	*	*	*	*	*	*	*	*
AS7	84.5	1.8	82.1	0.7	87.5	0.0	81.4	0.5	AS7	86.1	0.1	85.8	1.3	85.2	0.4	82.3	0.6
Mean	72.6	0.5	62.2	0.9	67.8	0.6	69.3	0.7	Mean	68.1	0.1	58.1	0.6	57.6	0.3	59.9	0.3
StDev	13.2	0.7	15.0	1.1	10.8	0.8	8.4	0.7	StDev	11.7	0.1	15.9	0.5	15.1	0.4	14.2	0.3
Twitch 2		***t to P***		***t to P***		***t to P***		***t to P***			***t to P***		***t to P***		***t to P***		***t to P***
AS1	19.3	49.7	15.8	46.8	14.9	47.7	16.4	46.8	AS1	16.6	47.7	10.0	51.8	11.1	48.6	11.0	41.2
AS2	15.5	56.5	10.6	42.4	12.3	53.0	10.7	47.1	AS2	13.1	43.0	5.9	37.8	5.7	36.3	5.0	33.9
AS3	18.0	63.6	17.7	46.6	16.8	57.5	14.1	51.1	AS3	19.9	47.9	15.0	47.7	17.8	65.9	11.6	53.7
AS4	11.9	54.6	9.1	54.0	10.6	53.7	8.5	52.7	AS4	11.5	47.7	7.6	49.6	9.6	54.0	7.9	35.7
AS5	15.4	46.2	10.5	42.0	9.9	44.7	7.9	41.0	AS5	13.8	44.5	6.7	43.2	7.9	42.1	5.9	40.8
AS6	12.0	67.9	*	*	14.4	54.9	8.5	67.4	AS6	13.9	53.0	*	*	11.9	50.6	12.9	81.6
AS7	20.9	52.3	15.7	62.2	18.7	60.2	13.7	57.9	AS7	18.9	60.8	17.6	63.9	19.2	60.9	17.1	53.8
Mean	16.1	55.8	13.2	49.0	13.9	53.1	11.4	52.0	Mean	15.4	49.2	10.5	49.0	11.9	51.2	10.2	48.7
StDev	3.5	7.6	3.6	7.8	3.2	5.4	3.3	8.6	StDev	3.1	6.0	4.8	8.8	5.0	10.2	4.2	16.5

* Missing data point due to equipment failure.

Twitch 1 Peak Torque (Nm)

1-TIME, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	3	29.49236	18	4.55993	6.467723	**0.003671**
2	1	30.64045	6	7.705479	3.976449	0.093185
12	3	2.030748	18	1.089724	1.863543	0.171962

		{1} 11.34929	{2} 8.361786	{3} 9.326858	{4} 8.186714
0 hr	{1}		**0.008197**	0.092953	**0.005187**
24 hr	{2}	**0.008197**		0.637308	0.996327
48 hr	{3}	0.092953	0.637308		0.508018
96 hr	{4}	**0.005187**	0.996327	0.508018	

MVC Peak Torque (Nm)

1-TIME, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	3	223.7422	15	50.0908	4.466733	**0.019714**
2	1	595.8168	5	276.4176	2.155495	0.201991
12	3	31.2616	15	25.5082	1.225551	0.334903

		{1} 70.31992	{2} 60.16167	{3} 62.69792	{4} 64.59225
0 hr	{1}		**0.014845**	0.078622	0.237854
24 hr	{2}	**0.014845**		0.816285	0.443474
48 hr	{3}	0.078622	0.816285		0.91201
96 hr	{4}	0.237854	0.443474	0.91201	

Twitch 2 Peak Torque (Nm)

1-TIME, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	3	66.0827	15	2.628376	25.14202	**4.22E-06**
2	1	49.04159	5	10.473	4.682668	0.082773
12	3	1.270736	15	1.48942	0.853175	0.486449

		{1} 16.22475	{2} 11.85767	{3} 12.87200	{4} 10.80575
0 hr	{1}		**0.000218**	**0.000869**	**0.000187**
24 hr	{2}	**0.000218**		0.443944	0.413615
48 hr	{3}	**0.000869**	0.443944		**0.031799**
96 hr	{4}	**0.000187**	0.413615	**0.031799**	

MVC Interpolated Twitch (Nm)

1-TIME, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	3	0.394051	15	0.290333	1.35724	0.293713
2	1	1.310201	5	0.89118	1.470186	0.279476
12	3	0.022366	15	0.288842	0.077434	0.971235

Twitch 1 Time to Peak Torque (ms)

1-TIME, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	3	25.24072	18	74.55641	0.338545	0.797691
2	1	295.3207	6	104.6761	2.82128	0.144025
12	3	49.45595	18	25.0097	1.977471	0.153497

Twitch 2 - Time to Peak Torque (ms)

1-TIME, 2-CONDITION

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	3	37.80119	18	67.9955	0.555937	0.650807
2	1	122.4257	6	45.9703	2.663148	0.153818
12	3	27.14905	18	27.36419	0.992138	0.418862

APPENDIX 10

MUSCLE DAMAGE RAW DATA AND ANOVA TABLES

1 = TIME; 2 = CONDITION

Z-BAND STREAMING DATA

	Fast Arm			Slow Arm	
Subject	**Pre**	**Post**	**Subject**	**Pre**	**Post**
No. Moderate Z-band Streaming/mm^2					
AS1	0.00	2.62	AS1	0.00	0.44
AS2	0.00	0.90	AS2	0.00	0.32
AS3	0.00	2.49	AS3	0.00	0.68
AS4	0.00	1.42	AS4	0.00	0.57
AS5	0.00	1.54	AS5	0.00	0.56
AS6	0.00	1.86	AS6	0.00	1.05
AS7	0.00	2.94	AS7	0.00	2.60
Mean	0.00	1.97	Mean	0.00	0.89
StDev	0.00	0.74	StDev	0.00	0.79
No. Extreme Z-band Streaming/mm^2					
AS1	0.00	0.33	AS1	0.00	0.00
AS2	0.00	0.00	AS2	0.00	0.00
AS3	0.00	0.28	AS3	0.00	0.00
AS4	0.00	0.00	AS4	0.00	0.00
AS5	0.00	0.00	AS5	0.00	0.00
AS6	0.00	0.00	AS6	0.00	0.00
AS7	0.00	0.00	AS7	0.00	0.29
Mean	0.00	0.09	Mean	0.00	0.04
StDev	0.00	0.15	StDev	0.00	0.11

No. Moderate Z-band Streaming/mm^2

	Mean	Std.Dv.	N	Diff.	Diff.	t	df	p
POSTFAST	1.967643	0.739525						
POSTSLOW	0.886643	0.787065	7	1.081	0.666112	4.29366	6	**0.005128**

No. Extreme Z-band Streaming/mm^2

	Mean	Std.Dv.	N	Diff.	Diff.	t	df	p
POSTFAST	0.086329	0.148188						
POSTSLOW	0.041429	0.10961	7	0.0449	0.205716	0.577468	6	0.584626

APPENDIX 11

ATPASE HISTOCHEMISTRY PROTOCOL

ATPASE HISTOCHEMICAL 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 30μm/cut. Once muscle sample is exposed, reduce thickness of cut to 10μm.
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	$CaCl_2 2H_2O$	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_20 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	$CaCl_2 2H_2O$	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 $CaCl_2 \cdot H_2O$ 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 $CoCl_2.6H_2O$ 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% $CaCl_2.2H_20$ (Calcium Chloride) for 3 minutes at room temperature.

7. Rinse slides in distilled water 5 times.

8. Incubate slides in 2% $CoCl_2 \cdot 6H_20$ (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 Permount (Fisher SP15-100). Allow Permount 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 12

MHC GEL ELECTROPHORESIS PROTOCOL

MHC GEL ELECTROPHORESIS PROTOCOL

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*

I. Sectioning/Lysing Procedure:

1. 1 ml or 0.5 ml microcentrifuge tubes - labeled with permanent marker and placed inside the cryostat to cool. Do not use tape to label the tubes.

2. Cut 4-6 sections (20 microns thick) for MHC analysis.

3. Ensure the tissue is at the bottom of the tube.

4. Add approx. 250-500 microliters 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.

5. Close lid, mix in vortex and place in warm water bath (10 min at 60*C).

6. Quick freeze immediately in liquid nitrogen and store at approx –80°C.

II. Preparation for Gel Loading Procedure:

Part A: Preparation of Glass Plates

Use large Kimwipes for a cleaning surface.

1. Carefully inspect plates for scratches and chips. Ensure inner surface (in contact with gel) is free of imperfections. Wash plates with methanol and wipe dry with Kimwipes.
2. After plates have been cleaned, wash spacers with methanol, wipe dry and place them on the edges of plates. Secure plates together with clamps (finger-tighten) and without moving spacers. ENSURE plates are evenly secured.
3. Fit laminated rubber gaskets in the bottom of the casting stand.
4. Place plate/clamp setup into casting stand. Ensure casting stand is level.
5. Secure plates in casting stand with cams (black knobs).

Part B: Preparation of Separating Gel – 4-8% gradient

	4%	8%
Glycerol (Glycerin) G8773	7.92 ml	11.1 ml
Acrylamide/Bis 30.8% ProtoGel EC-890	3.1 ml	6.2 ml
d.d. H_2O	6.48 ml (2 x 3.24 ml)	---
1.5 M Tris (pH 8.8) T3253	6.0 ml (2 x 3 ml)	6.0 ml
10% SDS L3771	0.24 ml	0.24 ml
10% APS A3678	0.24 ml	0.24 ml
TEMED T9281	0.015 ml	0.015 ml

1. Add reagents to 25 ml graduated cylinder. Insert stir bar, cover cylinder with parafilm, invert a few times and mix on stir plate until fully homogeneous.
2. Degas gel solutions using vacuum for 10 min on ice. Degas 4% gel solution first to allow for extra mixing time of 8% gel solution.
3. Transfer degassed gel solution into beaker and keep on ice.

To Pour Separating Gel:

(Have extra pipette tips ready to switch between 4 and 8 % gel solutions.)

1. Mark 11.5 cm from the bottom of glass plates.
2. First, transfer 2 x 3.98 ml of 4% separating gel solution to gradient column.
3. Allow flow of 4% solution to 8% column (stop flow just before entering column).
4. Transfer 2 x 3.98 ml of 8% separating gel solution.
5. Open both valves and turn on peristaltic pump to feed solution through tubing.
6. Place tube in the center of glass plates and fill to 11.5 cm mark.
7. Place 0.1% SDS overlay and ensure total surface is covered.
8. Allow to gel to set for 60-90 min using a lamp to accelerate polymerization process. Check polymerization with unused portion of separating gel solutions.

Part C: Preparation of Stacking Gel – 4%

Glycerol (Glycerin)	5.63 ml
Acrylamide/Bis 30.8%	1.95 ml
d.d. H_2O	3.36 ml
0.5 M Tris (pH 6.8)	3.75 ml
10% SDS	0.15 ml
10% APS	0.15 ml
TEMED	0.015 ml

1. Add reagents to 25 ml graduated cylinder. Insert stir bar, cover cylinder with parafilm, invert a few times and mix on stir plate until fully homogeneous.
2. Degas gel solutions using vacuum for 10 min on ice.
3. Transfer degassed gel solution into beaker and keep on ice.

To Pour Stacking Gel:

1. After the separating gel has fully polymerized, prop up one end of the casting stand.
2. Use a vacuum apparatus and a fine-tipped pipette tip and remove the 0.1% SDS overlay.
3. After adding TEMED to the 4% stacking gel solution, use a 60 ml plastic syringe to take up the solution and transfer the gel solution through the centre of the glass plates to approx. 1cm below the top edge.
4. Insert comb and centre in stacking gel.
5. Allow to polymerize for 60-90 min using leftover gel solution as a guide.

Part D: Preparation of 10 % APS

1. Weigh out 0.2 g and add 2 ml. of d.d. H_2O. You will hear "cracking" noise when H_2O is added.
2. Keep on ICE.

Part E: Preparation of Running Buffer

1. Pour 3 L of distilled water into a 4L beaker
2. Add 4 g Lauryl Sulfate (SDS) L3771
3. Add 12.14 g TRIZMA Base T6066
4. Add 57.6 g Glycine G8898
5. Bring up to 4 L and mix thoroughly with stir bar.

III. Gel Loading Procedure

1. Thaw samples and add 3-4 drops of glycerol (to give weight to samples).

2. Load 7.5ul of sample to stacking gel wells.

3. Add in order: 10% SDS, 10% APS and TEMED. Note: Polymerization occurs upon adding TEMED, therefore transfer gel solution IMMEDIATELY.

4. Once stacking gel has polymerized, slowly remove comb and add bromphenol blue (B8026) stained running buffer to each well.

5. Remove unpolymerized gel solution from each well using fine-tipped pipette tip attached to P200 pipette.

6. Repeat.

7. Leave wells filled with bromphenol blue running buffer to prevent drying out.

8. Add 7.5 μL of lysed proteins into each well. Leave either the first or last well empty for reference. Clean micropipetter with distilled water between each loading.

9. After all the samples are loaded, attach blank plate opposite to glass plates on casting stand.

10. Fit slotted rubber gasket onto the upper buffer chamber. Place upper chamber on glass plates and blank. Transfer cams to the top hole to secure upper chamber.

11. Fill vertical unit box with running buffer to a height of 10.5 cm. Insert the head exchanger then upper chamber apparatus. Using a plastic rod, agitate buffer to release air bubbles.

12. Carefully transfer unit to the refrigerator, then fill the upper buffer chamber with running buffer to just below exposed electrode.

13. Fit lid on the upper chamber, plug electrodes and set voltage to 120 V and amperage should read between 8-10 mA.

14. Allow protein separation to take place for 19-21 hours. After 20 hours, check the leading edge (bromphenol blue line) to ensure appropriate migration (at least halfway through the separating gel).

IV. Preparation for Destaining Procedure

Part A: Preparation of Coomasie Blue solution

1. Pour 500ml of 50% Methanol in to 1L glass container
2. Add 2g of2% Coomasie (Brillant Blue B0149)
3. Add 30ml of 3% Acetic Acid
4. Bring to 1L with ddH_2O

Part B: Preparation of Destaining Solution

1. Pour 400ml of 20% Methanol into a 1L container
2. Add1 40ml of 7% Acetic Acid
3. Bring to 2L with ddH_2O

IV. Gel Removal and Destaining Procedure

1. To remove gel from glass plates, carefully use the flat edge of a weighing spoon to pry apart the plates. Slowly and gently pry along the stacking gel side. If gel sticks to one plate, use spacer to gently pull the gel off and into staining dish.

2. Add Coomasie Blue stain to the dish and allow staining to proceed for 30-45 min.

3. To destain, pour out staining solution into appropriate disposal container and add destaining solution.

4. Change destaining solution within a few minutes for the first 2-3 washes or until solution turns blue. After the third or fourth wash, destaining solution can be changed after an extended period of time (15, 30, 1h).

5. After destaining, check MHC bands on slide/transparency viewer. Top two bands are MHC IIa, and IIx followed by MHC I.

6. Quantify relative content using laser densiometry – expressed band intensities as a percentage of total (use blank area from gel as zero reference).