CHANGES IN MUSCLE PROTEIN SYNTHETIC RATE AND ULTRASTRUCTURE FOLLOWING RESISTANCE EXERCISE

CHANGES IN MUSCLE PROTEIN SYNTHETIC RATE AND ULTRASTRUCTURE FOLLOWING RESISTANCE EXERCISE

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To my parents,

whom embody the essence of this thesis:

"Growth can be achieved through commitment."

For my "growth", as a result of their commitment,

I thank and love them.

ABSTRACT

The purpose of this study was to correlate the extent of myofibrillar disruption with muscle protein synthetic rate (MPS) following an isolated bout of concentric or eccentric elbow flexor resistance exercise. Six strength-trained males performed 8 unilateral sets of 8 repetitions at 80% concentric 1RM. The absolute amount of work performed by each arm was controlled by having both the concentrically-exercised (CON) and eccentrically-exercised (ECC) arms lift or lower the same weight through the same range and magnitude. Biopsies from biceps brachii of each arm, extracted ~21 h post-exercise, were analyzed electron microscopically to quantify myofibrillar disruption. The severity of disruption was classified as focal (FOC), moderate (MOD), or extreme (EXT). MPS of both arms was calculated from the increment in L-[1,2-$^{13}C_2$]leucine abundance in biopsy samples relative to the mean plasma [1,2-$^{13}C_2$]-α-KIC enrichment at isotopic plateau using the primed-constant infusion technique over ~10 h so that the midpoint of the assessment period was ~24 h post-exercise. The severity of disruption was significantly ($P < 0.001$) greater in both the FOC (11.2%) and MOD (12.2%) compared to the EXT (1.6%) rating. Absolute disruption of fibers was significantly greater ($P = 0.007$) in the ECC (44.7%) as compared to baseline (BASE) samples (3.9%), obtained following 5 d where no arm

training had occurred. In addition, ECC samples showed ~40% greater total disruption than CON samples (44.7% vs. 26.7%). Despite this, a positive correlation ($r = 0.89$) was found between individual values for MPS and the percentage of disrupted fibers in tissue from the ECC but not CON arm. These findings indicate that, in strength-trained males, residual myofibrillar disruption from a previous training session is essentially repaired within 5 d, but that resistance exercise-induced muscle damage did not appear to provide the activating signal for elevating MPS between ~21-29 h post-exercise.

PREFACE

The format of this thesis differs from the traditional thesis format in that this thesis is presented in two chapters. Chapter I is a literature review related to the induction, mechanisms, and methodology used to assess resistance training-induced muscle hypertrophy and the associated muscle fiber injury, repair, and adaptation. Chapter II consists of the thesis research and is presented in a manuscript format similar to what would be submitted to a journal for publication.

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CHAPTER I

SKELETAL MUSCLE HYPERTROPHY

1.1 INTRODUCTION

It is well known that the quality and quantity of muscle proteins in adult skeletal muscle can be affected by exercise training (Roy et al., 1991). The plasticity of skeletal muscle, both structurally and functionally, is particularly evident when a comparison is made between highly trained strength and endurance athletes. The varied, and distinctive, adaptations that result, demonstrate the highly specific nature by which proteins adapt to the intensity, duration, frequency and pattern of contractile activity placed upon them (Saltin and Gollnick, 1983). Exercise-induced muscle hypertrophy is the normal adaptation to the functional overload imposed by a program of resistance training. However, this manifestation of muscle plasticity has received much less study than the physiological modifications brought about through aerobic training.

Skeletal muscle hypertrophy is the result of an increase in the size of the existing muscle fibers by increases in the cross-sectional area and number of myofibrils (MacDougall, 1986). These growth processes can only occur when muscle

protein turnover favours synthesis over degradation (Babij and Booth, 1988; Smith and Rennie, 1990; Waterlow et al., 1978). The mechanism that regulates this has not yet been identified. The production of mechanical tension by muscle is thought to be the primary stimulus for growth (MacDougall, 1986; McDonagh and Davies, 1984; Vandenburgh, 1987), but what is still unknown is the signal that links the development of tension with increases in muscle protein synthesis.

It has been shown that both acute (Biolo et al., 1995; Chesley et al., 1992) and chronic (Rennie et al., 1980; Yarasheski et al., 1990a, 1993a, 1995) resistance training increases muscle protein synthesis in humans. Additionally, muscle tissue damage, inherent in these resistant exercises, has been well documented, especially in activities which contain large negative work components (i.e., eccentric muscle actions) (Evans, 1992; Fridén and Lieber, 1992; Gibala et al., 1995; Paul et al., 1989; Staron et al., 1992; Stauber et al., 1989). An eccentric muscle action (EMA) occurs when the muscle lengthens while actively producing force (Knuttgen and Komi, 1992). The damage that occurs, as evidenced through morphological disruption of fibers (Fridén et al., 1981, 1983a; Gibala et al., 1995; Newham et al., 1983a), the appearance of muscle enzymes in the blood (Clarkson et al., 1986; Hikida et al. 1991; Fridén et al., 1989; Schwane et al., 1983), changes in force production (Ebbeling and Clarkson,

1989; Gibala et al., 1995; Newham et al., 1983b), and delayed-onset muscle soreness (Asmussen, 1956; Newham, 1988; Schwane et al., 1983) is not permanent. The repair process, which may take up to 10-14 days (Jones et al., 1986; Manfredi et al., 1991; O'Reilly et al., 1987) is believed to be a normal precursor to muscle adaptation (Armstrong, 1984). This is supported by the finding that one bout of eccentric work protects against soreness and enzyme release during a second bout (Balnave and Thompson, 1993; Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Nosaka et al., 1991), and that the muscles appear more resilient against future damage following regeneration (Byrnes et al., 1985; Clarkson et al., 1992). Experimental evidence suggests that mechanical stress is a dominant factor for producing exercise-induced muscle damage (Kuipers, 1994). This is supported by the finding that the initial damage occurs at the site of the cross-bridge linkages, the Z disc and cytoskeleton (Fridén et al., 1983a, 1984; Waterman-Storer, 1991).

These observations, combined with the evidence which indicates an increase in muscle protein synthesis following resistance exercise (Biolo et al., 1995; Chesley et al., 1992; Rennie et al., 1980; Yarasheski et al., 1990a, 1993a, 1995), suggest that muscle damage may somehow stimulate an increase in muscle protein synthesis so that damaged fibers are repaired and made less susceptible to further insults. The

concept of muscle damage being the link between the increase in muscle protein synthesis brought about by mechanical tension was the focus of this project.

The purposes of this introductory chapter are: 1) to review the resistance training methods used to induce muscle hypertrophy in humans and animals, and the molecular and cellular signals that foster this response; 2) to evaluate the applicability of stable isotope methodology for the study of muscle protein synthesis in humans following resistance training. 3) to outline: a) the energetics and sites of injury associated with EMA; and b) the proposed model of the damage, repair and adaptation process. Finally, the importance of EMA, muscle damage, growth factors and satellite cell activation in the hypertrophy response will be discussed.

1.2 RESISTANCE TRAINING-INDUCED MUSCLE HYPERTROPHY IN HUMANS

Information on the cellular adaptations in human skeletal muscle from strength training is restricted to the analytical methods employed. Muscle biopsy samples can provide data on fiber type, fiber area and metabolic properties. Muscle-scanning techniques such as ultrasound, computed axial tomography scans and magnetic resonance imaging can measure muscle cross-sectional area.

Muscle fiber hypertrophy, in response to a functional overload, has been studied from both longitudinal resistance-

training studies and cross-sectional comparisons between experienced strength-trained athletes and sedentary control subjects. Collectively, the accumulated data indicate that a program of heavy resistance training can cause: 1) large increases in muscle strength due to neural adaptations, 2) large increases in muscle strength due to hypertrophy of type I and type II muscle fibers, with preferential hypertrophy of type II muscle fibers, 3) increases in high-energy phosphate stores of muscle, 4) increases in total content of connective tissue, and 5) no change or a decrease in muscle capillary density (MacDougall et al., 1977, 1979; MacDougall, 1986; Sale, 1992; Tesch, 1987).

While muscle fiber hyperplasia (an increase in fiber number) has been observed in several animal species following experimental protocols that induce muscle hypertrophy (Alway, 1991; Alway et al., 1993, 1989b; Gonyea et al., 1986; Ho et al., 1980), evidence that this occurs in adult humans is either indirect (Alway et al., 1989a; Tesch and Larsson, 1982, 1986; Tesch, 1987), or lacking (MacDougall et al., 1984; Saltin and Gollnick, 1983). For a more detailed discussion of this topic the reader can consult two reviews (MacDougall, 1992; Taylor and Wilkinson, 1986).

The hypothesis that high levels of mechanical tension in muscle are necessary for growth (MacDougall, 1986; McDonagh and Davies, 1984; Vandenburgh, 1987) is supported by the

common finding that contractile forces of greater than ~66% of the one-repetition maximum (1RM) are generally considered necessary in order to achieve muscle hypertrophy and strength gains (McDonagh and Davies, 1984). The greater relative hypertrophy of fast twitch (FT) over slow twitch (ST) fibers (Houston et al., 1983; MacDougall et al., 1979; Tesch et al., 1987; Thorstensson et al., 1976) will only occur when this threshold intensity is reached. The recruitment threshold for FT motor units is high, requiring loads to exceed 66% of the 1RM in order to affect muscle size and strength (Sale, 1992). In addition, the basal muscle protein synthetic rate in tonic muscles (soleus), consisting primarily of ST fibers, is greater than that of phasic muscles (lateral gastrocnemius), consisting primarily of FT fibers (Goldberg, 1967). Smith and Rennie (1990) provide support for this observation in humans, in which tibialis anterior (a predominantly ST muscle) was shown to have a greater basal protein synthetic rate than the quadriceps (with a more heterogenous fiber composition). These findings suggest that FT fibers may be more sensitive to perturbations than ST fibers.

The following review of resistance training studies illustrates the effectiveness of these training regimens for increasing the strength and mass of the major muscle groups of the body. Longitudinal studies of ≤6-mo duration have resulted in variable increases in muscle cross-sectional area and

strength. Elbow flexor strength and cross-sectional area increased by 92% and 23%, respectively, following 100 days of isometric training (Ikai and Fukunaga, 1970). Smaller increases in isometric elbow flexor strength (14.5%), and elbow flexor cross-sectional area (5.4%) were reported after a 6 week training study (Davies et al., 1988).

MacDougall et al. (1977) reported an 11% increase in arm circumference and 28% increase in maximal elbow extension strength after a 5-mo isotonic strength training program. Using a similar 5-mo period, subjects in MacDougall's study demonstrated increases of 33-39% and 27-31% for type II and type I fiber areas, respectively, in triceps brachii (MacDougall et al., 1979, 1980). Cross-sectional areas of the upper arm, determined by computed tomography scanning, were increased by 16% and 23% in men and women, respectively, after a 16 week progressive strength training program for the elbow extensors and flexors (Cureton et al., 1988). Absolute strength gains were significantly higher in males than females, but the percentage increases were similar, ranging from 33-42% for elbow extensors and 36-59% for elbow flexors. Following 5 weeks of unilateral leg extension isotonic strength training Young et al. (1983) found a 6% significant increase in quadriceps cross-sectional area using ultrasound scanning. Similar findings using elderly males were reported by Frontera et al. (1988). In this study the 12 weeks of

isotonic training elicited a 9.3% increase in the cross-sectional area of the quadriceps muscle group. Houston et al. (1983) demonstrated increases of 21% and 18%, respectively, for type IIa and type IIb fiber areas following 10 weeks of unilateral isotonic strength training of the knee extensors. Following a 20 week heavy resistance training regimen Staron and colleagues reported significant hypertrophy in female vastus lateralis muscle fiber areas (1989). Specifically, type I, IIA and IIAB + IIB fiber types increased by 15%, 45% and 57%, respectively.

Subjects involved in 60 days of unilateral isokinetic leg extension training increased the cross-sectional area of their quadriceps by 8.5% (Narici et al., 1989). Using the same training velocity as the previous study, Housh et al. (1992) reported significant hypertrophy of extensor and flexor muscles of the forearm and leg in males. Cross-sectional areas determined by magnetic resonance imaging, increased by 17.7% and 14.4%, respectively, for forearm extensors and flexors, and 8-34% and 15-30%, respectively, for selected leg extensors and flexors. Additionally, significant increases in isokinetic strength occurred for forearm extensors (36%) and flexors (21%) and leg flexors (22%).

These studies indicate that: 1) the magnitude of muscle hypertrophy is variable and independent of gender; 2) the growth process is relatively slow, 2) increases in muscle size

do not solely account for increases in strength, and 3) muscle hypertrophy can be achieved by employing isotonic, isometric, or isokinetic resistance training protocols.

Although these training studies elicited increased muscle cross-sectional areas in a range of 5.4-34%, observations from elite bodybuilders indicate a much greater magnitude of muscle enlargement. Cross-sectional data from MacDougall et al. (1984) revealed a 76% greater muscle cross-sectional area (measured by computerized tomographic scanning) in biceps brachii of bodybuilders compared to the normal population. Additionally, the strength trained athletes in a study conducted by Alway et al. (1988) exhibited a 2.5 fold greater mean fiber area of the gastrocnemius compared to an age-matched sedentary control group. Comparisons between male bodybuilders and untrained men revealed both a 57% greater elbow flexor cross-sectional area, determined by computerized tomographic scanning, and mean fiber area of the biceps brachii muscle in bodybuilders (Sale et al., 1987). In addition, female bodybuilders (Alway et al., 1989a) had a 31% greater biceps brachii cross-sectional area, than untrained females (Sale et al., 1988).

It has been suggested that there may be an upper limit for cross-sectional fiber and myofibril areas in muscles that have undergone hypertrophy (see section 1.9.1). Mean fiber areas of the triceps brachii in elite bodybuilders and

powerlifters were similar to a 6-mo, previously untrained, strength trained group in spite of large differences in elbow extension strength and arm girth (MacDougall et al., 1982). Additionally, 24 wk of heavy resistance training failed to elicit further increases in either muscle cross-sectional area or mean fiber area of the biceps brachii in highly competitive male and female bodybuilders (Alway et al., 1989a). These findings are consistent with those of Häkkinen et al. (1987, 1988) in which muscle size and strength did not change over a 2 year period in a group of highly competitive weight lifters. To account for the differences in muscle cross-sectional area without the concomitant increase in fiber size several theories have been proposed and reviewed recently by MacDougall (1992).

1.3 METHODS TO INDUCE MUSCLE HYPERTROPHY IN ANIMALS

To study the tension-induced hypertrophy of animal skeletal muscle a number of *in vivo* models have been employed. These include tenotomy (Denny-Brown, 1961; Goldberg et al., 1975), passive stretch (Alway, 1993, 1994; Holly et al., 1980; Vandenburgh, 1987), and weight lifting exercise (see below). Generalization of findings from such models to the human model is contingent upon the parallel between mechanical loading imposed by such techniques and the progressive overload involved with resistance training in humans. (For a comparison

of animal and human models used to induce muscle enlargement refer to Timson, 1990). For this reason only the weight lifting exercise model will be discussed in this section. However, the use of various animal models should serve to advance our understanding of the hypertrophy process. Furthermore, as newer *in vitro* techniques are developed to more closely simulate *in vivo* conditions, a more complete understanding of the signal that links the development of tension with increases in muscle protein synthesis may be possible.

1.3.1 Weight Training

Relatively few studies have been performed on animals using a resistance training protocol. Goldspink and Howells (1974) showed significant increases in mean fiber areas from 17.7% to 35.6% in biceps brachii of male hamsters. These animals underwent 5 weeks of volitional progressive training in order to obtain food rewards by pulling down on a counterweighted food basket. Similar increases in the mean fiber areas of the extensor digitorum longus were also found.

Wong and Booth (1988) used a model of resistance exercise consisting of non-voluntary electrical contraction of rat skeletal muscle. After 16 weeks of chronic (4 sets of 6 reps, 2 sessions per week) concentric resistance training with progressively increased weights, wet weight of the

gastrocnemius muscle of the trained leg was 18% greater than the contralateral control muscle. In a follow up study (1990a) they examined the acute and chronic effects of a similar resistance training design on protein synthetic rates in gastrocnemius muscle in rats. Following a single bout of concentric resistance training (4 sets of 6 reps/500 g) no significant change occurred in either mixed or myofibrillar protein synthetic rates compared to control (0 reps/0 g) animals. However, two additional groups, high frequency-low resistance (32 sets of 6 reps/0 g) and high frequency-high resistance (32 sets of 6 reps/800-1 100 g), showed similar significant increases of 38% and 47% in myofibrillar and mixed protein synthetic rates, respectively, at 12-17 h post-exercise. An interesting finding from both high frequency chronically trained groups was no significant change in gastrocnemius wet weight compared with the non-trained contralateral control muscle after 10 weeks of training. In explaining this disparity, Wong and Booth (1990a) cite the observation that simultaneous strength (high intensity) and endurance (high duration) training may hinder development of strength (Hickson, 1980). That is, additional protein regulatory responses (i.e., protein degradation) may be activated by high frequencies of concentric resistance exercise, which may outweigh the ability of high resistance exercise to stimulate a net increase in protein synthesis.

Using the same chronic training regimen, the eccentrically trained tibialis anterior muscle from the same set of animals was enlarged 16-30% compared with sedentary control animals (Wong and Booth, 1990b). In contrast to the concentrically-trained gastrocnemius, mixed and myofibrillar protein synthetic rates were elevated after acute exercise by 32% and 45%, respectively, for the moderate frequency-moderate resistance (4 sets of 6 reps/500 g) eccentrically trained tibialis anterior at 12-17 h post exercise. Additionally, both high frequency groups were statistically similar to this group with respect to the increase in mixed and myofibrillar protein synthetic rates (41 and 51% respectively) at 12-17 h post-exercise. These results indicate a differential response in both the acute changes in protein synthesis, and the regulation of protein accumulation following chronic training between rat gastrocnemius and tibialis anterior muscles. This conclusion may be attributed to a greater relative resistance imposed on the tibialis anterior than on the gastrocnemius (Wong and Booth, 1990b).

Klitgaard (1988) trained rats to lift a lever to exercise the plantar flexors. Over the 36 week training period the resistance on the lever rose by 85%. This progressive training regimen was reflected in a 24 and 34% increase in plantaris and soleus muscle wet weight, respectively. Garner et al. (1991) used a positive reinforcement protocol to motivate rats

to perform weight-lifting exercise. At the beginning of the 10 week training period animals were fitted with a weighted jacket corresponding to 5-10% of their body mass. In order to gain intracranial self-stimulation (positive reinforcement) rats were required to stand on their hind legs and press a bar positioned 200 mm above the cage floor. Weight-training sessions consisted of 20 weighted repetitions (> 35 seconds/rep) conducted 5 days/week. At the beginning of each subsequent week the resistance was progressively increased with some animals eventually training at resistances in excess of 50% of their body mass. Significant increases in both gastrocnemius wet weight (9.5%) and mean fiber diameter (33%) were found at the end of the 10 week training period. The smaller than expected increases compared to previous studies (Klitgaard, 1988; Wong and Booth, 1988) may be explained by the difference in training duration.

In a recent study by Linderman et al. (1994) resistance exercise was shown to prevent the normally occurring atrophy of the fast-twitch plantar flexors in rats with hindlimb suspension. In this experiment, rats climbed a 1 m 85° inclined ladder with an additional 50% of their body mass suspended from their tails. Training consisted of 3 daily bouts of 10 repetitions over 5 days. Consistent with these findings, Herbert et al. (1988) reported that four daily bouts

of 8 repetitions over 7 days with an additional 75% of the rat's body mass attenuated the relative but not absolute loss of mass of the suspended medial gastrocnemius.

In a similar ladder climbing study non-suspended rats underwent an 11 week training regimen (3 session/week, 8 repetitions/session) in which the tail-suspended weight increased from 20 to 100% of body mass (Deschenes et al., 1994). Resistance training evoked a significant 16.5% hypertrophic response in the soleus muscle compared to control. A similar 8% increase in rectus femoris wet weight was found by Yarasheski et al. (1990b) in rats performing 8 weeks of progressive weighted ladder climbing.

1.4 MOLECULAR CONTROL OF MUSCLE GROWTH

The adaptive transformations in protein composition of skeletal muscle which are induced by acute and chronic changes in contractile activity, suggest that this process is a primary regulator of muscle size (Roy et al., 1991; Smith and Rennie, 1990). Determining how contractile activity modulates the expression of muscle-specific genes is becoming an increasingly researched area in exercise physiology. Control of muscle gene expression occurs at the level of transcription and translation, in addition to the less well understood changes in the rate of protein degradation (Babij and Booth, 1988; Smith and Rennie, 1990).

In eukaryotic cells, a strand of DNA that codes for a specific protein (the coding strand) is transcribed or copied into an unedited message known as heterogeneous nuclear RNA (hnRNA) in the nucleus. The hnRNA transcript is modified and exported from the nucleus to the cytosol in the form of messenger RNA (mRNA). Protein biosynthesis, or translation, takes place at the ribosome in three steps (i.e., initiation, elongation and termination) and requires mRNA, transfer RNA (tRNA), ATP, GTP, amino acids and several protein factors (Campbell, 1991).

A variety of control mechanisms appear to exist in cells for the regulation of protein synthesis. These are: 1) transcription of DNA, 2) processing of hnRNA, 3) transport of mRNA across the nuclear membrane, 4) mRNA stability in the cytosol, 5) translation of mRNA, and 6) post-translational modification of the nascent protein (Babij and Booth, 1988; Nevins, 1983).

Indices of gene transcription and translation are reflected by changes in the RNA capacity and activity, respectively. Protein synthetic capacity (RNA capacity) can be defined as the total RNA concentration expressed relative to non-collagenous protein content. Since 80% of total RNA is ribosomal RNA (rRNA), a high correlation exists between muscle protein synthetic rates and total RNA content, which is reflected in this index (Waterlow et al., 1978). Translational

efficiency (RNA activity) is expressed as the protein synthetic rate relative to the total RNA content. Increases in this index imply that the rate of mRNA translation by the ribosomal machinery is accelerated due to changes in the rates of peptide chain initiation and/or elongation (Waterlow et al., 1978).

Signals arising from changes in the magnitude and pattern of contractile activity exert their effects at different stages during the expression of muscle genes (Babij and Booth, 1988). Acute changes in muscle protein synthesis seem to occur as a result of altered rates of translation in the cytosol (Barnett et al., 1980; Bylund-Fellenius et al., 1984; Chesley et al., 1992; Goldspink, 1977; Goldspink et al., 1983; Laurent et al., 1978; Wong and Booth, 1990a, 1990b). Additionally, it is generally found that no significant change in protein levels occur in the muscle with an isolated bout of exercise (Booth and Thomason, 1991).

Contrary to these findings, chronically altered contractile activity is associated with changes in protein levels and similar directional changes in the level of corresponding mRNAs (Brownson et al., 1988; Howard et al., 1989; Underwood and Williams, 1987; Williams et al., 1986; Wong and Booth, 1988, 1990b). This suggests that pretranslational and/or transcriptional control become

increasingly important during chronic alterations in contractile activity.

Increases in DNA content have also been observed following a chronic weight-lifting protocol in rats (Wong and Both 1990a, 1990b). Increased DNA in adult skeletal muscle cannot be from mature muscle contractile fibers. Evidence suggests that the source of the new DNA must be from satellite cell activation, connective tissue cell proliferation or infiltrating cells (Darr and Schultz, 1987).

1.5 REGULATION OF MUSCLE PROTEIN SYNTHESIS BY CELLULAR SIGNALS

The growth response of muscle to various forms of exercise has been well documented and is also known to be influenced by a number of genetic, nutritional, and hormonal factors (Vandenburgh, 1987). This section will discuss cellular signals thought to be involved in the stimulation of muscle protein synthesis. It should be recognized that the isolated influence of any one of these signals on its own may not be significant, however it is the complex interaction of these factors which ultimately determines the size of a muscle (Vandenburgh, 1987). In addition, section 1.12.2 summarizes the influence of muscle damage (the focus of this thesis) on growth factor and satellite cell activation in the regulation of muscle protein synthesis.

1.5.1 Amino Acids

Dietary amino acids entering the free amino acid pool are metabolized in a variety of pathways, resulting in either gains or losses of protein by or from the organism. It has also been proposed that dietary amino acids regulate many of the pathways involved in protein turnover, especially protein synthesis (Millward and Rivers, 1989). Among the amino acids that may have a regulatory role in muscle protein turnover are leucine, isoleucine, valine (the branched-chain amino acids), and glutamine. Of the 3 branched-chain amino acids, leucine appears to be the most potent regulator of muscle protein turnover. Despite the considerable body of literature on the influence of leucine on incubated or perfused skeletal muscle (see Millward and Rivers, 1989; Tischler et al., 1982), it has been difficult to demonstrate a direct influence of leucine on muscle protein balance *in vivo* in rats (McNurlan et al., 1982) or humans (May and Buse, 1989; Tessari et al., 1985).

In spite of this, the direct influence of glutamine as a potential regulator of muscle protein turnover has been observed in both rats and humans. The concentration of glutamine, the most abundant amino acid in muscle, is very sensitive to any insult which influences protein synthesis, degradation, and balance in muscle (Smith and Rennie, 1990). A positive correlation between intramuscular glutamine concentration and the fractional muscle protein synthetic rate

has been demonstrated in rats (Jepson et al., 1988; MacLennan et al., 1987; Rennie and Scislowski, 1988) and humans (Rennie and Scislowski, 1988) during states of increased intracellular glutamine concentration, protein deficiency and starvation. The mechanism for glutamine's regulation of protein synthesis is unknown, but it has been suggested that it may regulate the levels of its corresponding aminoacyl-tRNA synthase (Tyobeka and Manchester, 1985).

1.5.2 Changes in Cellular Energy Levels

Investigations of the relationship between muscle protein synthesis and changes in cellular energy levels suggest that phosphocreatine (PCr) splitting and changes in the ATP/ADP ratio of the cell serve as regulators of muscle protein synthesis. Since both protein synthesis and the excitation-contraction coupling of contractile activity are energy requiring processes, it follows that if both processes derive their energy sources from a common energy pool, then any contractile activity may impair the ability to synthesize protein.

Parallel decreases in the muscle protein synthetic rate and cellular energy levels (i.e., ATP and PCr content and the ATP/ADP ratio) of tibialis anterior, gastrocnemius and plantaris muscles were found by Bylund-Fellenius et al. (1984) following 10 min of electrically stimulated isometric muscle

actions. Similar findings in the electrically stimulated extensor digitorum longus muscle were noted by Pain and Manchester (1970).

Alternatively, a substrate or product from a metabolic reaction may influence muscle protein synthesis. Heavy exercise is known to reduce the muscle cell ATP/ADP ratio and to increase the cellular free P_i levels (Kemp et al., 1992). Phosphorylation is an important intracellular signalling mechanism and has been shown to play a role in the regulation of protein synthesis (Rana and Hokin, 1990). When initiation factor eIF-2 (a substrate for protein kinases and phosphatases) is phosphorylated, protein synthesis is decreased (Pain, 1986). This shows that acute down-regulation of muscle protein synthesis can be accomplished by perturbations in the muscle's energy level and its metabolic byproducts.

1.5.3 Prostaglandins

Prostaglandins are a group of small molecules, synthesized from membrane phospholipids and classified as hormones since they bind to specific intra- and extracellular receptors (Campbell, 1991). They are effective regulators of several physiological responses, and have been proposed as a modulator of muscle protein turnover. Two members of the

prostaglandin family, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2), are thought to affect the rate of protein synthesis and protein degradation respectively (Reeds et al., 1987). Studies have demonstrated a close temporal relationship between changes in muscle protein turnover and the release of their respective prostaglandins. Protein synthesis in rabbit forelimb muscle and $PGF_{2\alpha}$ release were increased by 70% and 105%, respectively, following 90 min of intermittent stretch (Palmer et al., 1983). In contrast an initial period of increased protein degradation and PGE_2 release was found with intermittent stretch of avian myoblasts (Vandenburgh et al., 1990). The exact nature of how mechanical tension stimulates the series of reactions synthesizing $PGF_{2\alpha}$ and PGE_2, and their effect on muscle protein turnover are poorly understood.

1.5.4 Hormonal Influences

It has been repeatedly demonstrated that heavy resistance exercise can induce both acute and chronic increases in the concentrations of anabolic hormones such as testosterone and growth hormone (for reviews see Kraemer, 1992; Yarasheski, 1994). However, the acute increases in anabolic hormone levels are probably not the primary stimulus responsible for increases in muscle protein synthesis and muscle hypertrophy

induced by heavy resistance training. This conclusion is based on the facts that: 1) work-induced hypertrophy can occur in hypophysectomized and castrated animals (Goldberg et al., 1975); 2) muscle hypertrophy is confined only to those muscle groups that have been resistance-trained, whereas increases in endocrine hormone levels would be expected to affect all muscle groups; 3) supraphysiological doses of growth hormone do not appear to enhance muscle protein synthesis in healthy humans (Copeland and Nair, 1994; Smith and Rennie, 1990; Yarasheski et al., 1990a, 1992a, 1993b, 1995). Nonetheless, Häkkinen et al. (1988) have suggested that chronic elevations in testosterone and the testosterone/cortisol ratio, evident in experienced strength trained athletes, may provide a more optimal anabolic environment for muscle growth. Continued research is needed to elucidate the direct, but more likely indirect role of hormones in inducing muscle hypertrophy.

1.6 STABLE ISOTOPE METHODOLOGY

The most common method for measurement of muscle protein synthetic rate (MPS) in humans is the tracing of a labelled essential amino acid and its incorporation into skeletal muscle. The labelled amino acid is normally introduced by either the; 1) primed-constant infusion or 2) flooding dose technique. In recent years the technique of choice has been the primed-constant infusion (Rennie et al., 1994). However,

Garlick and colleagues (1989; Essén et al., 1992; McNurlan et al., 1991) have continually used the flooding dose technique for its relative ease of application and the ability to make repeated measurements. The use of these techniques has relied on the technological advances in equipment (i.e., gas chromatograph/mass spectrometer) used to measure the fate(s) of several biological molecules through the application of stable isotopes. This section will: 1) outline the basis of stable isotope methodology, 2) review the strengths and weaknesses of each method, and 3) present data concerning MPS in humans in response to an isolated bout of resistance training in order to justify the use of stable isotope methodology following resistance training.

Prior to discussing the stable isotope methodology a review of some terminology encountered in the literature may prove helpful. Stable isotopes of a given element are defined as atoms having the same number of protons but differing in the number of neutrons. A stable isotope does not undergo spontaneous radioactive decay and can be found in varying amounts in nature. The most common stable isotopes used to label amino acids are ^{13}C and ^{15}N and their natural abundances are approximately 1.11% and 0.37%, respectively (Wolfe, 1984).

The rate of protein synthesis can be theoretically calculated from a measurement of the change in the incorporation of a labelled amino acid into the protein and the precursor pool enrichment (i.e., the isotopic enrichment of the tracer at the site of protein synthesis). The labelled amino acid is normally made available to the cellular machinery of protein synthesis by infusion or injection into the venous circulation. This increases the plasma and body pool enrichments of the label above natural background levels. The increase in enrichment is usually expressed in atom percent excess (APE). Kinetic factors that identify the rate of appearance of a labelled amino acid in the plasma pool (Ra) and the rate of its disappearance (Rd) from plasma into tissue are more frequently used in the "forearm model" of protein metabolism (Cheng et al., 1985, 1987).

Several assumptions must be met by any method used to determine protein turnover. The following assumptions have been identified and supported by Waterlow et al. (1978) and Wolfe (1984). These are: 1) the labelled tracer is indistinguishable biologically from the naturally occurring molecule, 2) the administration of a tracer dose of the label will not disturb the kinetics of the system, 3) the tracer is distributed uniformly and rapidly in all the body pools, and 4) during a continuous infusion protocol measurements of leucine kinetics are made only when an isotopic steady-state

exists (Tessari et al., 1985; Thompson et al., 1989; Wolfe, 1984).

In the primed-constant infusion method, a stable isotopically labelled (e.g., ^{13}C) tracer amino acid is infused. This involves the injection of a bolus dose (usually 1 mg/kg of a 99% ^{13}C labelled amino acid dissolved in normal saline) followed by a constant rate of infusion of the label for several hours (1 mg/kg/h). The bolus dose is used to achieve an isotopic steady-state quickly by rapidly elevating the enrichment of the plasma and tissue pools. An isotopic steady-state is achieved when the rate of appearance (Ra) of the label in plasma is equal to its rate of disappearance (Rd) into tissue (Wolfe, 1984). Only when Ra=Rd can accurate measures of whole body protein turnover and muscle protein synthesis be made. If Ra<Rd protein synthesis will be overestimated while if Ra>Rd protein synthesis will be underestimated. The duration of the infusion of the tracer amino acid need only be as long as the time needed to reach this isotopic steady-state, and to obtain a measurable increase in enrichment of the target proteins. If the measurement period is too long, however, this can lead to isotope recycling (Schwenk et al., 1985). Isotope recycling is a process where a labelled amino acid is incorporated into protein and subsequently released during the infusion period.

This would lead to loss of label from the tissue and an underestimation of the MPS. Fortunately, the slow turnover rate of myofibrillar proteins exceeds the infusion time for a primed-constant infusion of a labelled amino acid such as leucine (i.e., 6-10 hours) (Rennie et al., 1994).

Calculation of the absolute rate of protein synthesis depends upon knowledge of labelling of the true precursor pool (i.e., the appropriate aminoacyl-tRNA pool). One of the major drawbacks of the primed-constant infusion protocol is its inability to measure the enrichment of the true precursor pool because of the large tissue sample required and the tedious and elaborate procedure involved in its isolation and purification (Garlick et al., 1989). The flooding dose technique permits an indirect estimate of the precursor pool enrichment by making measurements of the isotopic enrichment of the tracer amino acid in plasma. This is possible because the isotope is given together with a large amount (i.e., ~3-4 g) of the unlabelled amino acid that causes rapid isotopic equilibration of the free extracellular and intracellular amino acid pools (Jahoor et al., 1992). The intracellular amino acid pool includes the precursor pool for amino-acyl-tRNA for protein synthesis (Wolfe, 1984). Therefore, from a theoretical standpoint, precursor pool enrichment can be accurately estimated by analysing plasma (Garlick et al., 1989; McNurlan et al., 1991). The rate of protein synthesis is

then calculated by comparing the incorporation over the period of the application of the flood with the average plasma amino acid labelling (Rennie et al., 1994).

Extensive human and animal research has been aimed at measuring the labelling of this precursor pool in muscle (Baumann et al., 1992; Chikenji et al., 1983; Watt et al., 1991; and/or see Table 1 Rennie et al., 1994). From these data it is seen that the aminoacyl-tRNA labelling is generally found *in vivo* to be intermediate between the intracellular and plasma amino acid labelling. However, researchers have sought to use a surrogate index of the labelling of the aminoacyl-tRNA due to the rapid turnover rate of these complexes (2-10 seconds) and the large tissue requirements (Smith and Rennie, 1990; Waterlow et al., 1978). This led to the adoption of leucine as a tracer and measuring the labelling of the transamination product of leucine, α-ketoisocaproate (α-KIC) as the aminoacyl-tRNA surrogate index (Horber et al., 1989; Matthews et al., 1982; Thompson et al., 1988).

Leucine is a branched-chain amino acid that is oxidized primarily in muscle by branched-chain amino acid transaminases (Odessey and Goldberg, 1972). Upon entry into muscle leucine is rapidly transaminated to α-KIC in a reversible reaction catalyzed by aminotransferase (Campbell, 1991). Three reasons underlie the use of α-KIC as a surrogate of the true precursor labelling. First, "being produced intracellularly, it should

accurately reflect the extent of labelling in the true precursor pools for protein synthesis and amino acid oxidation" (Rennie et al., 1994). This was demonstrated in a study by Watt et al. (1991) in which the labelling of α-KIC after tracer infusion of leucine came within 10% of the labelling of leucyl-tRNA in human skeletal muscle. Second, α-KIC readily traverses the muscle cell membrane allowing for rapid equilibration between intracellular and extracellular α-KIC concentrations. Third, plasma α-KIC can be isolated and measured by gas chromatography/mass spectometry (Ford et al., 1985; Rocchiccioli et al., 1981).

Smith and Rennie (1990) and Thompson et al. (1989) have outlined major considerations in the choice of an amino acid, its label and label position, and the infusion doses and times. These are: 1) the amino acid concentration gradient between plasma and muscle, 2) the intracellular free amino acid pool size, 3) the concentration of amino acid bound in protein, and 4) the cellular metabolism of the amino acid. Leucine is an appropriate tracer when studying MPS because it optimizes these criteria. First, leucine's free intracellular concentration in muscle is only 0.41% of the total free amino acid pool while its concentration in muscle protein is 8-10% of the total bound amino acid pool (Smith and Rennie, 1990). By maximizing the gradient between the free intracellular leucine concentration and its protein bound concentration in

muscle the efficacy of using leucine as the tracer amino acid becomes apparent when considering the slow turnover rates of the myofibrillar proteins (Waterlow et al., 1978). It is easier to label a small free amino acid pool than a large pool. Additionally, once incorporated into the muscle, the release of label is minimized by the slow turnover of muscle protein.

An additional fate of leucine, once incorporated into the muscle cell, is an irreversible decarboxylation at the α-carbon to yield isovaleryl CoA. Thus, the infusion of singly or doubly (L-[1-^{13}C] or L-[1,2-$^{13}C_2$]) labelled leucine permits the recovery of the label in expired CO_2 to measure leucine oxidation and in muscle protein obtained from biopsy samples to measure MPS. The absolute rate of protein synthesis can be calculated from the increase in incorporation of ^{13}C leucine in muscle samples obtained several hours apart. As mentioned previously this calculation should be based on the enrichment of the tracer at the site of protein synthesis (i.e., the appropriate aminoacyl-tRNA, or suitable surrogate). This is reinforced by the findings of Schneible et al. (1981), that labelling of intracellular leucine pools may not be uniform due to compartmentation. This introduces the term, "fractional muscle protein synthetic rate", expressed as a percentage of protein synthesized per hour (%/h). The equation used in the

calculation of the MPS is the absolute change in leucine incorporation (in APE) divided by the product of plasma α-KIC enrichment (in APE) and the incorporation time between 2 biopsies (hours). This is multiplied by a factor of 100 to obtain a value expressed in %/h.

A consistent finding, when comparing the primed-constant infusion method with the flooding method, is an almost 2 fold increase in the rate of muscle protein synthesis in the latter (Rennie et al., 1994). Attempts have been made to resolve this controversy. For a more thorough explanation the reader is directed to the following references (Jahoor et al., 1992; Rennie et al., 1994; Smith et al., 1992a, 1992b). In brief these data strongly suggest that the flooding dose protocol is causing an increase in the rate of incorporation of tracer into protein. In effect this disqualifies this technique based on the second criterion proposed by Waterlow et al. (1978) and Wolfe (1984) (see above). In the review by Rennie et al. (1994), it is further proposed that the flooding dose causes a supraphysiological effect which increases the incorporation of the labelled amino acid into protein without any change in the precursor labelling. This is supported by the findings that leucine increases the stability of aminoacyl-tRNA (Morgan et al., 1985), and the failure to observe an increase in the proportion of polyribosomes formed despite an increase in

incorporation of tracer into muscle protein (McNurlan et al., 1991).

Recently the originators of the flooding dose method have used [$^2H_5$]phenylalanine as the flooding amino acid (Calder et al., 1992). This was in response to the observation that leucine has been implicated in stimulating muscle protein synthesis *in vitro* (Tischler et al., 1982) and slightly *in vivo* (Smith et al., 1992a; Wibert et al., 1991). These authors have found significantly lower values for protein synthesis using phenylalanine (0.067 %/h) (Calder et al., 1992) compared to leucine (0.088 %/h) (see Table 2 of Rennie et al., 1994). Typical values for mixed muscle protein synthetic rates, using the primed-constant infusion method range from 0.04-0.06 %/h (Rennie et al., 1994). In spite of the potential practical advantages of the flooding dose technique, such as a considerably shorter experimental period and the possibility of obtaining an accurate estimate of the precursor enrichment, the greater variability and absolute values obtained seem to favour the application of the primed-constant infusion method.

An increasing number of studies have measured MPS in humans in response to resistance training. Significant increases in vastus lateralis protein synthetic rate have continually been found following resistance training protocols ranging from 2 to 16 weeks (Rennie et al., 1980; Welle et al.,

1995; Yarasheski et al., 1990a, 1992a, 1993a, 1995). Recently, Biolo et al. (1995) found vastus lateralis protein synthetic rate to be significantly elevated 3 h after an acute bout of resistance exercise. Tarnopolsky et al. (1991) revealed no increase in whole body protein synthetic (WBPS) rate for up to 2 hours following an isolated bout of circuit resistance training. However, caution should be exercised with this interpretation since Nair et al. (1988) found that MPS account for only 27% of the WBPS rate in spite of muscle contributing 40-50% of total body weight. These data stress the importance of measuring MPS directly since WBPS rates do not give quantitative information about the anabolic state of muscle. This is further supported by the findings of Fryburg and Barret (1993). To determine the time course for elevated MPS MacDougall and colleagues examined three time points following an isolated bout of elbow flexor resistance training. Results indicated that biceps brachii protein synthetic rate was significantly elevated by 50%, 109%, and non-significantly by 14% at approximately 4, 24, and 36 h post-training, respectively (Chesley et al., 1992; MacDougall et al., 1992, 1995). Collectively, these data indicate the applicability of the use of stable isotope methodology following an isolated bout of resistance exercise. Furthermore, data from Chesley et al. (1992) and MacDougall et al. (1995) indicate, for biceps brachii, MPS reached a peak at approximately 24 h post-exercise.

1.7 THE PHYSIOLOGY OF ECCENTRIC MUSCLE ACTION

Physical activity consists of varying combinations of concentric, isometric and eccentric muscle actions. Since most forms of human movement seldom involve only one of these actions, Knuttgen and Komi (1992) have used the term "eccentrically-biased exercise", to distinguish exercise models that contain large negative work components (e.g., downhill running, eccentric cycle ergometry). Observations on the physiological differences between these muscle actions began in the early 1900's from experiments on stair climbing/descending or by lifting and lowering weights (Asmussen, 1953). Asmussen established that negative work (eccentric muscle action) was both energetically less demanding than an equivalent load of positive work (concentric muscle action) (1953), and produced substantially greater muscle soreness (1956). Continued research in this field has verified and attempted to explain these early findings.

1.7.1 Energetics

Early research by Abbott et al. (1952) and Asmussen (1953), revealed that the oxygen cost of submaximal eccentric cycling between 50-70 rpm is about 15% less than that of concentric cycling at the same speed and that this difference became greater (i.e., up to 30%) as the speed of cycling increased. Similar differences in metabolic efficiency using

cycle ergometry (Hesser et al., 1977; Knuttgen et al., 1971) or downhill walking (Davies and Barnes, 1972) have been reported by others. More recently Dudley et al. (1991a) compared the energy cost of concentric muscle actions to exercise that involved both concentric and eccentric muscle actions. Their subjects performed supine leg press resistance exercise, and the results indicated that performing both muscle actions (effectively doubling the exercise volume) added little to the energy cost (~14%) of the exercise.

The markedly lower oxygen cost of eccentric muscle actions is thought to reflect the fact that less motor unit involvement is required to maintain the same force output, and the energy cost per unit of force development is less during muscle lengthening than shortening (Stauber, 1989). This is supported by studies of isolated muscle preparations, where the energy requirement of a fiber (measured from its rates of ATP and phosphocreatine breakdown, and metabolic heat production) was much lower when the fiber was stretched compared to when it was shortened (Curtin and Davies, 1975).

Using integrated electromyographic (IEMG) activity, which reflects the recruitment and frequency of action potentials in a muscle fiber (Basmajian, 1978), Bigland-Ritchie and Woods (1976) examined differences in positive and negative work on a motorized cycle ergometer. They demonstrated that, under comparable, workloads there was less IEMG activity and a

smaller oxygen uptake during eccentric cycling. In addition, the reduction in oxygen consumption was substantially greater than the decrease in IEMG, providing support for the previous observation (Curtin and Davies, 1975) that active muscle fibers require less oxygen when they are being stretched than when they are shortened. Newham et al. (1983b), also used IEMG activity to demonstrate that fewer motor units were recruited in the eccentrically acting leg during a stepping task as compared to the concentrically acting contralateral leg. The authors concluded that since greater tension per muscle fiber is generated during the eccentric condition, fewer fibers need to be recruited, but are producing relatively larger forces.

The ability to perform the same work rate with less neural input is due to the inherent capacity of skeletal muscle to develop greater force during eccentric than concentric or isometric actions (Stauber, 1989). The functional significance of this is demonstrated in the finding that eccentric exercise has consistently been established as the most damaging in both humans (Fridén et al., 1983a; Gibala et al., 1995; Newham et al., 1993a) and animals (Armstrong et al., 1983a; Ogilvie et al., 1988).

1.8 INITIAL EVENT IN MUSCLE FIBER INJURY

Muscle damage and delayed onset muscle soreness (DOMS), primarily resulting from eccentrically-biased exercise

are well documented (Armstrong, 1990; Evans and Cannon, 1991). Little is known, however, about the initiating pathophysiological mechanisms, although loss of intracellular Ca^{2+} homeostasis seems integral (Armstrong, 1990). The primary changes involve the myofibrils and cytoskeleton (Fridén et al., 1983a, 1984; Waterman-Storer, 1991), and it is hypothesized that metabolic or mechanical stress is the potential initiating factors (Kuipers, 1994).

The metabolic stress hypothesis seems to focus on a compromised microcirculation, which could induce changes leading to high temperature, insufficient mitochondrial respiration, oxygen free radical production, and lowered pH level within the muscle. The mechanical stress hypothesis is supported by the finding that the initial damage occurs to the contractile apparatus. Additionally, although metabolically less demanding than concentric muscle actions, greater myofiber injury occurs with eccentric muscle actions, presumably due to the higher mechanical stress per fiber.

1.8.1 Metabolic Hypothesis

A) High Temperature

Evidence from myopathic conditions, such as malignant hyperthermia, have implicated high muscle temperatures as a stimulus for muscle damage due to the associated severe

degeneration of muscle fibers (Ebbeling and Clarkson, 1989). The mechanism for this insult, whether in disease or exercise, is an elevated muscle temperature, damaging the structural proteins, particularly the contractile apparatus of the muscle fibers (Armstrong, 1984). In addition, Davies and Barnes (1972) and Nadel et al. (1972), have reported higher local temperatures with eccentric muscle actions compared to concentric muscle actions at the same workload. Although metabolically less demanding, the higher muscle temperatures with eccentric exercise are likely the result of a lower rate of heat removal as predicted by the Fenn effect (Armstrong et al., 1991). It should be noted that within a muscle's physiological temperature range (24-42°C) muscle protein degradation rates are proportional to increases in muscle temperature (Armstrong, 1990).

B) Insufficient Mitochondrial Respiration

Attenuated ATP levels, due to insufficient mitochondrial respiration, could compromise the ATP-dependent Ca^{2+} pumps, effectively raising cytoplasmic Ca^{2+} levels (Armstrong et al., 1991). The deleterious effects of elevated Ca^{2+} concentrations was evaluated by Duncan (1987). Incubation of chemically skinned fibers in physiological Ca^{2+} concentrations (0.5-8 μmol/L) stimulated characteristic patterns of muscle damage

(i.e., Z-line streaming, dissolution and breakdown of the myofilaments). *In vivo* experiments with rats have confirmed the relationship between elevated Ca^{2+} concentrations and muscle fiber damage (Duan et al., 1990).

Heavy exercise is known to reduce the muscle cell ATP/ADP ratio due to an imbalance favouring ATP hydrolysis over synthesis (Hellsten-Westing et al., 1993; Kemp et al., 1992). However, owing to the fact that eccentric muscle actions are less metabolically demanding yet cause more fiber damage, most researchers contest this hypothesis as a viable explanation. Interestingly, both of the previously mentioned studies found perturbations of muscle bioenergetics without concomitant enzymological evidence of muscle damage. Nonetheless, Armstrong et al. (1991) contend that focal lesions within the fiber may have a metabolic origin (i.e., localized areas of ATP depletion).

C) Oxygen Free Radical Production

The ability of muscle tissue to rapidly change its energy supply and oxygen flux during exercise predisposes it to oxygen free radical-mediated damage (Jackson and O'Farrell, 1993). Free radicals are molecules or molecular fragments containing an unpaired electron in the valence shell. In general, oxygen radicals and the reactive species which they

generate alter, either directly or indirectly, the size, shape, and activity of compounds (i.e., enzymes, membrane structures) they interact with (Jackson and O'Farrell, 1993). Free radical attack on skeletal muscle tissue can occur when their production exceeds that of the body's free radical defence system (i.e., scavenger enzymes and antioxidant systems). Cell membrane function and integrity are jeopardized by a process called lipid peroxidation (Sjödin et al., 1990). Davies et al. (1982) reported that exercise to exhaustion in rats resulted in increased lipid peroxidation and free radical generation concomitant with indices of muscle damage. Similarly, a single bout of exhaustive running induced an oxidative stress response that was proportional to the amount of morphological disruption in the rat skeletal muscle fiber (Duarte et al., 1993). Support for this relationship in humans was provided in a study by Maughan et al. (1989) in which a 45 min. bout of downhill running produced both the classic muscle fiber injury and elevated free radical generation. However, the metabolic changes that accompany endurance exercise (e.g., body temperature, substrate availability, oxygen consumption, hormone status) may be responsible for the increase in free radical production observed (Ebbeling and Clarkson, 1989).

Studies using isolated muscle tissue subjected to excessive isometric and concentric contractile activity have

revealed an activation of both Ca^{2+}-dependent degradative pathways and oxygen radicals (Jackson and O'Farrell, 1993). Where damage to skeletal muscle specifically induced by eccentric muscle actions has been studied, conflicting data exist. In one study, stimulated lengthening muscle actions in mouse extensor digitorum longus muscle produced losses in maximum isometric force production and muscle damage (Zerba et al., 1990). However, treatment with superoxide dismutase (a free radical scavenger) attenuated these changes, suggesting oxidative damage could contribute to the initial injury. Studies of human subjects undertaking eccentric exercise have reported changes in blood parameters indicative of increased (Jackson and O'Farrell, 1993; Jakeman and Maxwell, 1993) and no change (Saxton et al., 1994) in free radical activity. Researchers advise caution when interpreting these results, owing to the insensitivity and unspecificity of plasma markers in evaluating free radical-mediated muscle damage (Saxton et al., 1994).

D) Lowered pH

Experimental evidence from both human and animal investigations do not support the hypothesis that muscle damage following exercise is due to elevated $[H^+]$. This is based on the findings that significantly less lactate is

produced from eccentric, than comparable concentric muscle actions, while incurring greater fiber damage and DOMS (Armstrong et al., 1983a, 1983b; Schwane et al., 1983).

1.8.2 Mechanical Hypothesis

Eccentric, as compared to concentric or isometric, muscle actions distribute the applied forces over a smaller cross-sectional area of muscle (i.e., the tension per active cross-sectional area is greater) (Stauber, 1989). It seems probable, therefore, that the greater fiber injury common to eccentric muscle actions is a result of this increased tension per unit area causing mechanical disruption of structural elements in the muscle fiber. Indeed, the most common disruption in muscle damage studies occurs to the contractile apparatus, especially the Z disc (see reviews by Evans and Cannon, 1991; Fridén, 1984a). In addition, the mechanical forces experienced during active muscle lengthening could potentially damage the sarcolemma, sarcoplasmic reticulum (SR), basal lamina, or surrounding connective tissue (Stauber, 1989).

Recently, Lieber and Fridén (1993) have proposed that it is not high force per se that causes muscle damage after eccentric actions, but the magnitude of the active strain (i.e., strain during active lengthening). Strain is defined as the amount of deformation divided by the original length of the structure. In a series of experiments that imposed cyclic

length changes of identical magnitude and velocity, contractile and morphological properties were measured in the rabbit tibialis anterior muscle (Lieber and Fridén, 1993). In the first experiment the muscles were strained 25% of the fiber length (L_f) at a strain rate of 125% L_f/s. The only difference between groups was the timing of the stretch. In the early stretch (ES) group, the muscle was stretched coincident with the onset of muscle activation. In the late stretch (LS) group, muscle stretch was delayed for 200 ms while the muscle developed tension. Although the stretch magnitude and rate were the same between groups, due to the timing of the stretch the peak force reached in the LS group was significantly higher than that of the ES group. In the second experiment the same protocol was repeated at one-half the total strain (i.e., 12.5% L_f). Combining the results of both experiments, high and low strains were imposed at high and low forces to test the effect of force and strain independently. Results indicated that large differences in force during eccentric muscle actions did not result in muscle damage differences, as evidenced by identical contractile and morphological characteristics. However, large strain differences did result in contractile and morphological differences. Lieber and Fridén (1993) concluded that muscle damage is the result of the muscle fiber strain that occurs

during lengthening of an activated muscle (i.e., active strain). However, they did caution interpretation of the data citing various potential difficulties as well as supporting further research involving sarcomere length measurements with laser diffraction.

As previously indicated, elevated intracellular Ca^{2+} could result from a variety of alterations in cell function and morphology induced by exercise. Calcium homeostasis has also been implicated in the mechanical hypothesis. Damage to the sarcolemma or SR system, brought about by severe lengthening of the sarcomere, could disrupt the normal Ca^{2+} sequestering ability of the cell (Armstrong et al., 1991). In this situation the fiber could enter a rigor-like state (Stauber, 1989), exacerbating the damage response for adjacent fibers (Tidball and Daniel, 1986). A further role of elevated intracellular [Ca^{2+}] may be to stimulate Ca^{2+}-sensitive enzymes (e.g., proteases, phospholipases) which are active in fiber degeneration (Byrd, 1992).

1.9 STRUCTURAL EVIDENCE OF MUSCLE DAMAGE

The variety of exercise models used to induce muscle damage have continually shown structural evidence of muscle damage to the contractile apparatus. It seems that the Z disc is the weak link in the chain of contractile units, since it

is most often reported to be damaged in needle biopsy samples (i.e., broadened, smeared, out of register, or totally disrupted) (Fridén et al., 1981, 1983a, 1988, 1992; Gibala et al., 1995; Newham et al., 1983a; Staron et al., 1992). Z band streaming is a non-specific indicator of muscle damage. Although it is often seen in patients with neuromuscular disorders (see references 6-19 in Meltzer et al., 1976), it has been reported in a small percentage (~2-7%) of the fibers examined from healthy, non-training individuals (Gibala et al., 1995; Meltzer et al., 1976). While the exact cause of Z band streaming following eccentric muscle action is unknown, disruption of the myofibrillar cytoskeleton has been implicated (Fridén et al, 1984; Waterman-Storer, 1991). This section will review the ultrastructure of the Z disc and cytoskeleton, and then focus on studies using eccentrically-biased exercise to induce myofibrillar disruption.

1.9.1 Ultrastructure of the Z Disc and Cytoskeleton

Skeletal muscle possesses a highly ordered and repeated pattern of structurally specialized protein filaments. The contractile units (sarcomeres), complete with thick, thin and intermediate filaments, are bounded by the Z discs. Before the introduction of the electron microscope the Z disc was described as either a membrane which traversed the muscle fiber, or as a fenestrated structure through which the

myofibrils passed (Knappeis and Carlsen, 1962). It is now considered as a platelike protein structure that secures the actin filaments and various intermediate filaments (Sheterline, 1983). Z discs give muscle fibers their characteristic striated appearance in longitudinal section, corresponding to the dark narrow line which bisects the I-band between adjacent sarcomeres. When viewed at high resolutions with the electron microscope, the Z disc appears as a jagged zig-zag structure, with thin filaments projecting from the points formed on either side (Pollack, 1990). In cross-section these thin filaments form the corners of the square lattice appearance of the Z disc (Figure 1). Cross-sections taken through the A-band reveal a hexagonal lattice appearance of the thin filaments. Goldspink (1992) considers this mismatch between the square and hexagonal lattice as a mechanism for his earlier observation of myofibril splitting (1971). Specifically, when tension is developed, mechanical stress is realized at the centre of the Z disc resulting from the oblique pull of the actin filaments. The ensuing rip in the Z disc is experienced along the myofibril resulting in two daughter myofibrils (Goldspink, 1992). Rowe and Morton (1971) provided support for this so called upper limit on myofibril size when they reported fault lines in the square lattice, predisposing these as sites of rupture. The consequence of the existence of such faults and ruptures may be interpreted as a

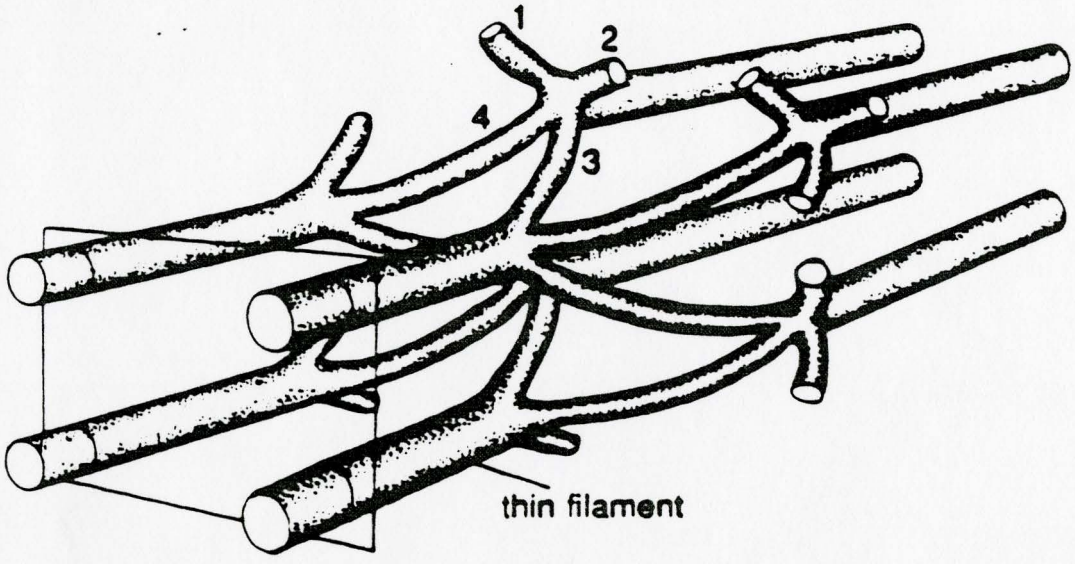


Figure 1. Schematic model of the Z disc (Pollack, 1990; after Knappeis and Carlsen, 1962). Intersecting plane illustrates the square lattice formed by 4 thin filaments.

lowering of the ability of the Z disc to withstand internal stress, which increases during growth or as a result of resistance training (Goldspink, 1992). This may partially explain the increase in myofibrillar number observed in the latter situation (see section 1.2).

Although the precise organization and chemical composition of the Z disc are not known, one of the associated proteins (α-actinin) has been identified. Alpha-actinin, being more prevalent in slow fibers, is an actin-binding protein that holds this filament in place and in register (Billeter and Hoppeler, 1992).

In addition to the contractile filaments (myosin and actin) it was realized that some noncontractile elements must be present to hold the sarcomere together. This was confirmed in experiments where both actin and myosin were extracted, yet the residual Z discs maintained their regular longitudinal spacing (Huxley and Hanson, 1957). This structural regulatory system has been termed the myofibrillar cytoskeleton. It is composed of protein filaments that interconnect to form a structural network throughout the sarcomere (Figure 2), and provide the actual physical framework for muscle actions (Waterman-Storer, 1991). Wang (1985) has designated two sets of cytoskeletal elements. The exosarcomeric cytoskeleton, consists primarily of the intermediate filament (IF) desmin. Desmin is localized to the Z disc region and contributes to

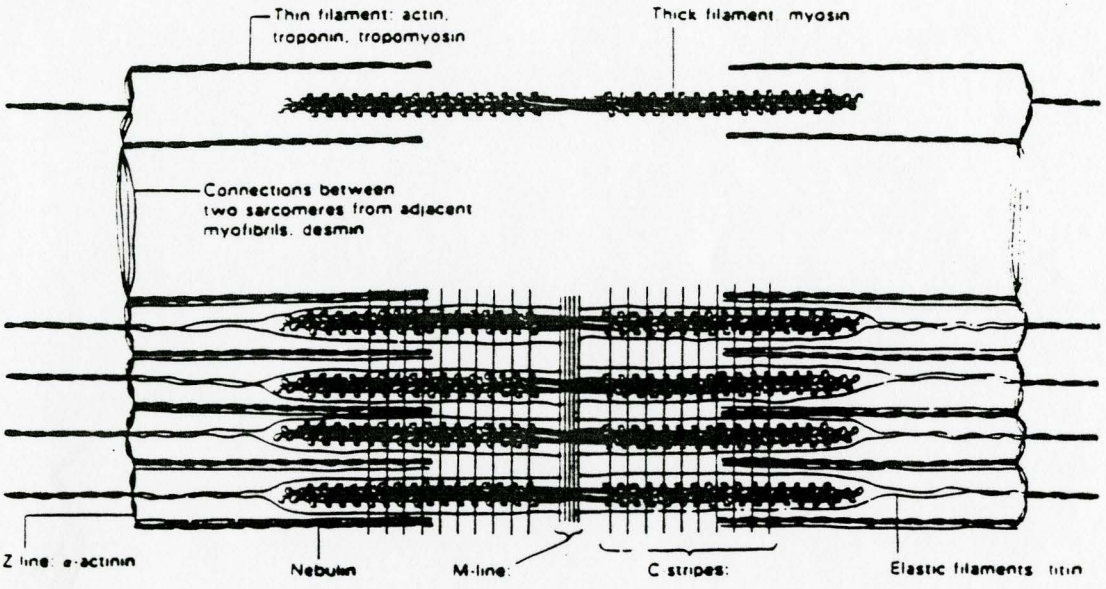


Figure 2. Schematic representation of the cytoskeletal proteins in the sarcomere (Billeter and Hoppeler, 1992).

the highly organized stacking, both transversely and longitudinally, of the sarcomeres at the Z disc (Tokuyasu et al., 1983). By linking adjacent Z discs, desmin maintains the lateral register of the sarcomere (Waterman-Storer, 1991). Wang and Ramirez-Mitchell (1983) isolated a longitudinally oriented IF (vimentin), originating from the periphery of successive Z discs, running parallel to the myofibril. By linking Z disc to Z disc, vimentin may have a mechanical role in limiting extreme length changes within individual sarcomeres. In the same study these authors identified an IF (synemin) encircling the Z disc in a doublet structure. It was proposed that these doublets may be the site of Z disc splitting, and therefore play a role in muscle hypertrophy. Additionally, Fridén and co-workers (1983a, 1984, 1988, 1992) believe it is the exosarcomeric cytoskeleton that is altered by eccentrically-biased exercise and implicated in the cause of Z disc streaming (see section 1.9.2).

The second set of cytoskeletal elements are those of the endosarcomeric cytoskeleton. These include titin and nebulin, which respectively regulate the myosin and actin molecules contained in these filaments, and maintain the registry of the thick and thin filaments in sarcomeres that have been stretched beyond overlap (Billeter and Hoppeler, 1992; Wang, 1985). Determination of the location of titin has been accomplished by immunofluorescence and immunoelectron

microscopy. Nave et al. (1989) reported that the globular head of the molecule is anchored at the M-line, while the rod-like section attaches to the Z disc. The labelling pattern observed at different sarcomere lengths suggests that titin found in the I-band is elastic, while titin in the A-band is bound to the outside of the thick filaments (Horowits and Podolsky, 1987; Whiting et al., 1989). The accumulated data on the physiological role of titin, as cited in Funatsu et al. (1990), indicate that the principal role of titin is to give both longitudinal and lateral passive elasticity to the sarcomere. In addition it functions to keep the thick filaments in the center of the sarcomere during muscle actions. Unlike titin, nebulin is inextensible irrespective of sarcomere length (Wang and Wright, 1988). Nebulin appears to be rigidly bound to the Z disc, running parallel to the thin filament to the I-band region in relaxed muscle (Wang and Wright, 1988). Nebulin may also play a role in thin filament packing geometry, or help actin interdigitate with myosin in the A-band when the sarcomere is stretched beyond overlap (Waterman-Storer, 1991). The relationship of these endosarcomeric proteins with the pathology of exercise-induced muscle damage is unknown. However, based upon Fridén and Lieber's (1992) observation of decentralized myosin filaments with eccentrically-biased exercise, broken titin filaments, as a result of the high tension, seem a probable cause. That is,

selective breakage of titin on one side of the sarcomere would cause the elastically anchored myosin filament to shift from its equilibrium position in the sarcomere (Horowits and Podolsky, 1987). In contrast, it seems likely that nebulin filaments are unaffected by high intensity exercise as the packing of actin filaments have never been observed to be disturbed (Waterman-Storer, 1991).

1.9.2 Myofibrillar Disruption Following Eccentrically-Biased Exercise

Following 30 min of cycling on an ergometer modified for eccentric work, Fridén and colleagues have demonstrated myofibrillar (1983a) and cytoskeletal (1984) disturbances originating from the Z discs. In the former study, biopsies taken 1 h and 3 d after exercise revealed Z disc disruptions (marked streaming, broadening, total disruption) in 32% and 52% of the examined fibers, respectively. No clear pattern of disruption was evident, with some fibers having only one single Z disc out of register, while others involved several sarcomeres and myofibrils. In their follow-up study Fridén et al. (1984), using immunofluorescence microscopy, observed longitudinal extensions of cytoskeletal intermediate filaments between Z discs of one or more sarcomeres at only the 3 d post-exercise time point. The pronounced delay in myofibrillar and cytoskeletal disorganization may be explained by a number

of degradative factors which will be explained in section 1.11.

Ultrastructural evidence of myofibrillar damage from walking (Newham et al., 1983a) and running (Fridén et al., 1981) downstairs has also revealed disturbances originating from the Z disc. Newham et al. (1983a) had subjects perform a 20 min stepping task, in which the quadriceps of one leg acted concentrically, while the contralateral leg acted eccentrically. Bilateral biopsies were taken prior to exercise, immediately after, and 24-48 h later. The authors quantified the damage as (i) focal (a lesion which affected 1-2 adjacent sarcomeres or 1-2 adjacent myofibrils), (ii) extensive (a lesion which affected more than 2 adjacent sarcomeres and more than 2 adjacent myofibrils, or a fiber which contained more than 10 focal areas), (iii) very extensive (a fiber which contained more than 1 extensive area). Samples taken prior to exercise, or from the concentrically exercised leg showed no morphological deformities. Samples from the eccentrically exercised leg had disruptions in 40% of the fibers immediately after exercise (16% focal, 16% extensive, 8% very extensive). Samples obtained an average of 30 h post-exercise increased in both the absolute amount of disruption (57%), and the extent of their damage (6% focal, 23% extensive, 28% very extensive). Results from Fridén et al. (1981) are not as definitive with

respect to the delay in myofibrillar damage as biopsy sampling only occurred pre-, and 2 and 7 d post-exercise. However, the total area showing focal disturbances in tissue obtained 2 d post-exercise was estimated to be 3 times larger than in comparable sections obtained before and 7 d post-exercise.

1.9.3 Myofibrillar Disruption Following Resistance Exercise

Resistance exercises, by their nature involve distinct concentric and eccentric movements. Until quite recently (Gibala et al., 1995) this segregation, as it relates to muscle damage, had not been exploited. Staron and colleagues have investigated muscle damage following traditional 6-10 RM isotonic resistance training, which contains both concentric and eccentric muscle actions (Staron et al., 1989, 1991, 1992). Following a 20 wk heavy resistance training program for the lower extremities, light microscopy revealed evidence of myofiber degeneration (e.g., fiber necrosis, phagocytosis, monocyte infiltration) and regeneration (e.g., internal nuclei, small normal fibers) in 7 of the 24 women. To determine the combined effects of repeated biopsy sampling and resistance training on muscle morphology Staron et al. (1992) used an 8 wk progressive resistance training program by men and women. Biopsies were taken from vastus lateralis of both the experimental and control groups at the beginning and every two weeks during the study. With the exception of one

specimen, there was no evidence of ultrastructural damage in any of the initial samples. Subsequent biopsies showed evidence of fiber degeneration and regeneration in both groups, but the training subjects had an approximate four-fold greater number of damaged fibers as the nontraining subjects (9% vs 2%). Internal disorganization (e.g., Z line streaming, myofibrillar disruption) was only evident in the training subjects. It was therefore concluded that muscle damage as a result of the biopsy procedure was not completely repaired after two weeks. In addition, resistance training may delay the repair process as well as cause additional damage to the myofibril.

The only study, to my knowledge, to address the pure nature of the muscle action with its subsequent damage was conducted recently by Gibala et al. (1995). In this study 8 previously untrained males performed the concentric phase of arm-curl exercise with one arm and the eccentric phase with the other arm. Muscle ultrastructural properties were examined from bilateral biopsy samples taken before, immediately and 48 h after a single bout of resistance exercise (8 sets of 8RM). Only 3% of the samples taken prior to exercise revealed disruption. Samples extracted immediately post-exercise from the eccentrically exercised arm revealed significantly more myofibrillar disruption (82%) than those from the concentrically exercised arm (33%). The extent of fiber

disruption in their respective arms 48 h after exercise were similar to the findings immediately post-exercise (80% and 37%). In addition, based upon the employed rating scale, the arm that performed eccentric muscle actions showed more severely disrupted fibers at both post-exercise time points. These data clearly illustrate that eccentric muscle actions induce greater myofibrillar damage than comparable concentric muscle actions.

1.9.4 Fiber Type Involvement

The literature on eccentrically-biased exercise suggests that type II fibers are especially prone to injury. In a study employing 30 min of eccentric cycle ergometry, Z disc disturbances were predominately found in type II fibers by a ratio of approximately 3:1 over type I fibers, three days after exercise (Fridén et al., 1983a). Although no evidence for selective fiber recruitment, on the basis of PAS-staining was found in Fridén et al.'s (1983a) study, O'Reilly et al. (1987) performing a similar protocol reported substantial type II fiber involvement with this technique. In addition, incomplete glycogen repletion, focal loss of myofibrillar organization and Z disc material, and damage to the SR and mitochondria were noted up to 10 d following the exercise. Possible explanations for the preferential damage to type II fibers included the high tension demands and lack of glycogen

repletion associated with this form of exercise (Fridén et al., 1983a; O'Reilly et al., 1987). In addition, the commonly observed difference in Z disc width (i.e., type II fibers having the narrowest Z discs (Eisenberg, 1983)) was considered as a possible mechanism (Fridén et al., 1983a).

Using repetitive bouts of sprint running, Fridén et al. (1988) examined specific fiber type damage using an ultrastructural characterization design based on the number and width of M-bridges and Z band widths. Thirty-six percent of the vastus lateralis fibers examined exhibited morphological abnormalities 2 h following the exercise. The majority (80%) of these fibers were characterized as type IIb fibers. A significant metabolic demand was placed on these fibers, since PAS-stained sections of the same biopsies showed glycogen depletion in the type I and IIb, but not IIa fibers. It was proposed that differences in the architecture of the Z disc, compounded by a significant metabolic demand, place type II, and especially type IIb, fibers as being most vulnerable to repetitive, high tension stress (Fridén et al., 1988; Fridén and Lieber, 1992).

Lieber et al. (1991) examined the ultrastructural effects of metabolically similar isometric (I) and eccentric (E) muscle actions, and passive stretch (P) on rabbit tibialis anterior muscles. A 25% L_f strain was imposed in E and P

conditions. Ultrastructural abnormalities were absent in muscles from the I or P group, while a significant portion of the fibers in the E group displayed various degrees of disorganization of the sarcomeric band pattern (i.e., Z disc streaming and smearing, focal loss of Z disc, extension of Z disc into adjacent A-bands) analogous to those observed in human muscle after intense eccentrically-biased exercise (Fridén et al., 1981, 1983a). Moreover, myofibrillar damage was confined to the ultrastructurally defined (see above) type II fibers.

Fridén and Lieber (1992) have proposed a theory of muscle damage based upon the muscle's oxidative capacity. Briefly, it contends that once type II fibers become fatigued, their inability to regenerate ATP places them in a rigor or high-stiffness state. Subsequent stretch of stiff fibers would mechanically disrupt the fibers (see section 1.8.2). A second damage mechanism that could depend on fiber oxidative capacity relates to the other cellular processes that rely on oxidative metabolism (see section 1.8.1, especially "Insufficient Mitochondrial Respiration").

Alternatively, the observation that type II fibers generate higher specific tensions (i.e., force per cross sectional area) may provide an explanation for their greater damage. With the assumption that type II fibers correspond to fast motor units (Burke, 1981), Bodine et al. (1987) isolated

single motor units of the cat tibialis anterior. Each unit was classified as either i) fast, fatiguable (FF); ii) fast, fatigue-intermediate (FI); iii) fast, fatigue-resistant (FR); or iv) slow, fatigue-resistant (S). Specific tension was calculated by dividing the maximum tension of a unit by its total cross sectional area. They reported the overall mean specific tension of the fast motor units (FF, FI and FR combined) was significantly greater than that of the S units. Although this specific tension difference between fiber types has not been confirmed in human subjects (Miller et al., 1993; Schantz et al., 1983), the methods employed in these studies are much less precise than those used by Bodine et al. (1987).

1.10 ADDITIONAL SITES OF MORPHOLOGICAL DAMAGE

1.10.1 Sarcolemma

The maintenance of the ionic and electrical gradients between the interior and exterior of the muscle cell is performed by the sarcolemma. Disruption of this membrane has been implicated in the appearance of muscle proteins (particularly creatine kinase, CK) in the blood following eccentric exercise (Armstrong, 1990). Clarkson's group has shown large increases (20-25 fold over baseline values) in serum CK (Clarkson et al., 1986; Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990). These studies employed a series of maximal eccentric actions of the elbow flexors performed on

a pulley apparatus to induce damage (see review by Clarkson et al., 1992). A consistent finding in these studies is the extremely delayed time course for CK release. An increase in serum [CK] was not evident until 2 d following exercise, and usually reached a peak approximately 4 d post-exercise (Clarkson et al., 1992). It should be noted that the magnitude and time course of CK response has been examined after several forms of exercise (i.e., stepping exercise (Newham et al., 1983c); downhill walking/running (Balnave and Thompson, 1993; Byrnes et al., 1985; Schwane et al., 1983); marathon running (Hikida et al., 1983); and weight lifting exercises (Fridén et al., 1989; Hikida et al., 1991; Nosaka and Clarkson, 1992; Paul et al., 1989; Rodenburg et al., 1993; Tiidus and Ianuzzo, 1983)). Briefly, the amount of change and the time course after these exercises is markedly different. In addition, there is considerable inter- and intra-subject variability in the serum enzyme response to exercise. It seems that the extent of the post-exercise rise in circulating skeletal muscle enzymes is most closely related to the type and intensity of the exercise and the previous training status of the subjects (Balnave and Thompson, 1993). It is likely that the post-exercise rise in circulating CK is a manifestation of skeletal muscle damage, but until direct evidence of holes in the plasma membrane is presented theories on sarcolemmal disruption will remain hypothetical.

Such hypotheses have focused on the concept of physical disruption to the sarcolemma allowing Ca^{2+} to enter the cell across its electrochemical gradient (Armstrong, 1984; Newham et al., 1983a; Stauber, 1989). As indicated previously (see section 1.8.2), high levels of intracellular Ca^{2+} can rapidly lead to cell dysfunction through Ca^{2+}-activated degradative pathways (i.e., proteases and phospholipases) (Byrd, 1992). Following the initial injury, further degradation of the cell membrane by these compounds may explain the delayed time course for CK release (Clarkson et al., 1992).

1.10.2 Sarcoplasmic Reticulum

Regulation of the intracellular free [Ca^{2+}] is performed by the sarcoplasmic reticulum (SR). Disruption of the SR could cause the sudden increase in intracellular free [Ca^{2+}] that triggers the cascade of events resulting in cellular damage and muscle autolysis discussed previously. It is speculated that the high specific tensions (Armstrong, 1990; Ebbeling and Clarkson, 1989) or strain (Lieber and Fridén, 1993) associated with eccentric muscle actions may mechanically damage the SR.

Studies conducted by Byrd and associates have demonstrated altered SR function following exhaustive treadmill exercise in rats (Byrd et al., 1989a) and horses (Byrd et al., 1989b; McCutcheon et al., 1992). Based upon the

results of these experiments it was suggested that initial SR dysfunction was a result of influencing metabolic factors (i.e., increased muscle temperature, decreased pH, oxygen free radical production) (see review by Byrd, 1992). Although these mechanisms may be responsible for SR dysfunction following endurance exercise, data on SR function following resistance exercise, using the standard techniques employed in these experiments (isolated SR vesicles), is lacking.

1.10.3 Extracelluar Matrix

The extracellular matrix (ECM) of skeletal muscle, consisting of type IV and V collagen, fibronectin, laminin, and various proteoglycans, surrounds and interconnects individual myofibers. The ECM is anchored to the sarcolemma of each fiber by continuous thin mats of specialized ECM called basal laminae (Alberts et al., 1989).

Evidence of ECM disruption following eccentric muscle actions has been reported in both humans (Stauber et al., 1990) and rats (Fritz and Stauber, 1989). Needle biopsy samples taken 48 h after untrained subjects performed 70 maximal isokinetic eccentric actions of the elbow flexors revealed ECM disruption (Stauber et al., 1990). These disruptions included separation of the ECM from myofibers, mast cell degranulation and increased plasma constituents (e.g., albumin, fibrinogen) in the extracellular space. It was

proposed that the mechanical strain of the exercise damaged the ECM, initiating an inflammatory response (evidenced by the release of mast cell granules) causing swelling. There have been several studies investigating the interrelationship between acute inflammation following eccentric muscle actions and DOMS (for review see Smith, 1991). Newham (1988) has suggested that pain and inflammation follow a different time course than the indicators of muscle damage. Therefore, the specific processes that follow muscle injury such as tissue swelling and ECM disruption may be more important in the production of pain (i.e., DOMS) and inflammation than the mechanical damage to the myofiber (Stauber et al., 1990). This is supported by the fact that mast cell degranulation releases histamine, a known algesic.

1.11 SEQUENCE OF EVENTS IN EXERCISE-INDUCED MUSCLE FIBER INJURY, REPAIR AND ADAPTATION

The sequence and time course of events associated with exercise-induced muscle fiber injury are not known (Kuipers, 1994). This section summarizes a five stage model proposed by Armstrong et al. (1991). It should be noted from the outset that very little is known about the mechanisms underlying the first three stages of the model, and the processes involved in the final two stages appear similar to those following other

types of muscle injury (Armstrong et al., 1991; Carlson and Faulkner, 1983).

The first stage of the proposed model is the *initial event* that triggers the succeeding degenerative and regenerative phases of the injury process. Although the nature of the initiating event(s) is not known, several possibilities have been hypothesized and have been outlined in section 1.8. A common factor in the proposed initiating event is the loss of cellular Ca^{2+} homeostasis. Each of the initiating factors discussed could either lead to, or result from, elevated intracellular free [Ca^{2+}]. This induces the second (Ca^{2+} *overload phase*) and third stages (*autogenetic mechanisms*), which activate several degradative pathways intrinsic to the fiber (e.g., Ca^{2+}-activated neutral proteases, lysosomal proteases, phospholipases, and the cascade of degradative reactions initiated by these enzymes: see Figure 7 in Armstrong, 1990). The autogenetic mechanisms are activated at the local site of injury prior to, and continue after, the invasion of phagocytic and inflammatory cells into the injury site.

Although it is difficult to distinguish between the autogenetic and *phagocytic phase*, by 2-6 h after the injury, processes initiated and regulated by phagocytic cells (i.e., mononuclear cells from the blood) probably dominate the

progression of necrosis, and continue for several days. It is the action of this phase which presumably accounts for the common observation of delayed myofibrillar and cytoskeletal damage following eccentric exercise (see section 1.9.2). Additional evidence supporting delayed myofiber damage following eccentric exercise includes the delayed release of CK (see section 1.10.1).

Finally, the *regenerative phase*, beginning 4-5 days after the injury, restores the muscle to its normal or improved condition. Research into the restoration and adaptation of eccentrically exercised skeletal muscle generally infers that the adaptive response makes the muscle more resistant to damage from subsequent bouts of similar exercise.

Fridén and colleagues (1983a, 1983b), using an exhaustive eccentric cycle ergometer protocol, studied the adaptive responses in skeletal muscle ultrastructure in a series of experiments. They showed that a single bout of such exercise gave rise to pronounced muscle soreness, strength reduction, and myofibrillar damage in untrained subjects (Fridén et al., 1983a). Repeated bouts over a longer period of time (i.e., 4 or 8 weeks) significantly reduced all these deleterious outcomes (Fridén et al., 1983b). They suggested that the functional adaptations of the exercised muscles were a consequence of improved coordination and reorganization of the contractile apparatus. In addition, Fridén (1984b) observed

large differences in sarcomere lengths from the fibers predominately responsible for force generation under these circumstances (i.e., type II fibers). He suggested this observation to be a sign of sarcomerogenesis and structural alterations in the type II fibers, resulting in a better distensibility of the fibers, reduced risk for mechanical damage and inducing an optimal overlap between actin and myosin filaments.

Support for this contention was provided by an immunocytological study of the intermediate filaments (Fridén et al., 1984). The observed longitudinal extensions between successive Z discs were attributed to either (i) cytoskeletal damage induced directly by the high tension, or (ii) sarcomerogenesis, a secondary response to the initial injury. It has been suggested (Waterman-Storer, 1991) that following initial disruption of the intermediate filament cytoskeletal Z bridges, various cellular processes eventually prepare the damaged area for the insertion of new Z discs. Evidence of a non-lysosomal proteolytic pathway involving ubiquitin conjugated to abnormally folded or partially damaged proteins may be involved in these preparatory processes. Free ubiquitin in the cell can form complexes with abnormal proteins, marking them for further degradation by a protease recognizing ubiquitin and requiring ATP (Schlesinger and Hershko, 1988). Increased levels of conjugated ubiquitin have been observed in

human biceps brachii 48 h following high-force eccentric exercise (Reichsman et al., 1991; Thompson and Scordilis, 1994) and in damaged rat muscle following spaceflight (Riley et al., 1990, 1992). Ubiquitin-mediated proteolysis of damaged myofibrillar proteins is reinforced by the 3-7 times higher density of conjugated ubiquitin found at the Z disc (the most often reported site of muscle damage; see section 1.9) than anywhere else in the myofibril (Riley et al., 1988).

The muscle fiber oxidative capacity hypothesis proposed by Fridén and Lieber (1992) (i.e., type II fibers entering a rigor or high-stiffness state once fatigued; see section 1.9.4), may also explain the protective effect of eccentrically-biased endurance training on eccentric exercise-induced damage. Endurance training is known to result in an increased muscle oxidative capacity, and therefore a type IIb to IIa fiber subtype conversion (Roy et al., 1991). Because type IIa fibers do not fatigue and enter rigor as readily as type IIb fibers, eccentric exercise-induced damage could be expected to be lower following endurance training (Fridén and Lieber, 1992).

Using a 40 min, 6.4 km/h eccentric walk down a 25% gradient on a treadmill, Balnave and Thompson (1993) studied the effect of training on exercise-induced muscle damage. Control subjects performed the exercise only at the start and end of the experiment. Experimental subjects performed the

exercise once a week for 8 wk. Results showed that training reduced DOMS, serum indicators of muscle damage (CK and myoglobin), and muscle function impairment. Dick and Cavanaugh (1987) showed that the IEMG activity increased progressively during a bout of prolonged sub-maximal downhill running. It was concluded that this increased recruitment of motor units caused the upward drift in oxygen consumption. Armstrong (1984) has suggested that the increase in IEMG during a bout of eccentrically-biased exercise indicates that injury to initially recruited motor units may necessitate recruitment of additional units to produce the required force. However, Knuttgen et al. (1982) demonstrated that eccentrically-biased exercise training eliminated the upward drift in oxygen consumption during prolonged eccentrically-biased exercise. Because eccentric exercise training greatly reduces the amount of muscle damage caused by the exercise, the initial injury to the fibers would not occur, avoiding the need for increased recruitment of motor units.

A number of studies have been conducted using 70 serial repetitions of maximal effort eccentric actions of the forearm flexors. The criterion measures used in these studies include serum [CK], muscle soreness and pain questionnaires, isometric strength, and elbow joint angles, and were assessed up to 5 d following each exercise bout. Clarkson and Tremblay (1988) had 8 women perform 3 eccentric exercises of the forearm flexors.

One arm performed 70 maximal actions (70-MAX), and the other arm performed 24 maximal actions (24-MAX) followed 2 wk later by 70 maximal actions (70-MAX2). Significant changes in all criterion measures were found after the 70-MAX condition, with a slow recovery that was not complete 5 d after the exercise. Only small changes in the criterion measures were obtained in the 24-MAX condition. More importantly, changes in the criterion measures after the 70-MAX2 condition were significantly smaller than those after the 70-MAX condition, and had returned to baseline values by 3-4 d post-exercise. The authors concluded that an adaptation took place such that the muscle is more resistant to damage and can be repaired at a faster rate.

Ebbeling and Clarkson (1990) showed that a subsequent bout of 70 maximal eccentric actions either 5 or 14 d after the first bout resulted in significantly smaller changes in the criterion measures, and a faster recovery time. Interestingly, the group that performed the second bout 5 d after the first bout still had significantly elevated indicators of muscle damage. However, the second bout neither exacerbated these indicators nor changed the time course for muscle restoration. Similar findings were reported by Donnelly et al. (1992), where subjects performed 70 maximal eccentric actions followed 24 h later by a light bout of eccentric exercise (25 reps at 50% of the maximal torque of the initial

bout). The subsequent bout occurred 14 d after the initial session. These data suggest that an adaptive response had taken place prior to full recovery and restoration of muscle function following the initial eccentric exercise bout.

Nosaka et al. (1991) investigated how long the adaptation effect lasts following this exercise protocol. Their results indicated that these adaptations can last as long as 6 wk after the initial exercise session. Additionally, serum [CK] was significantly lower, compared to the initial bout, in subjects performing a second bout 10 wk later, and 6 mos following this second bout.

1.12 MUSCLE DAMAGE AND THE HYPERTROPHY PROCESS

1.12.1 Importance of Eccentric Muscle Actions in Resistance Exercise

The primary goals of most resistance training programs is to increase the strength and size of the exercised muscles. It appears that the performance of eccentric muscle actions (EMA) in these training programs optimizes the development of muscular strength (Colliander and Tesch, 1990a; Dudley et al., 1991b; Komi and Buskirk, 1972) and hypertrophy (Evans and Cannor., 1991; Hather et al., 1991). Dudley, Tesch, and colleagues in their respective laboratories (Colliander and Tesch, 1990a, 1990b, 1992; Dudley et al., 1991a, 1991b; Hather et al., 1991; Tesch et al., 1990a, 1990b) have extensively

examined the role of EMA in resistance training programs. Their approach has been to examine the effects of resistance training during isolated concentric actions. Typical of the findings in these studies are those of Dudley et al. (1991b), who showed that elimination of EMA in progressive, heavy resistance training compromised optimal strength development. In their study, 3 groups of subjects trained for 19 wk with 4-6 multiple sets of heavy resistance knee extension and leg press lifts. One group (CON/ECC) performed both the concentric and eccentric phases of the lifts as is normally done. A CON group trained with the same number of repetitions but performed only the concentric phase of the lifts. A double concentric (CON/CON) group also trained with the concentric phase only but performed twice as many repetitions as the CON group. Results demonstrated a similar hierarchy of rankings of strength improvement for both the leg press and knee extension exercises, such that CON/ECC>CON/CON>CON. Specifically, the increase in leg press 3 RM was significantly ($P \leq 0.05$) greater for the CON/ECC (26%) than either CON/CON (15%) or CON (8%) groups. The increase in leg extension 3 RM was significantly ($P \leq 0.05$) greater for the CON/ECC (29%) than the CON group (16%), but not the CON/CON group (24%). Additionally, these differences were still evident following 4 wk of detraining. The authors hypothesized that an enhancement of neural adaptations with EMA may be caused by increased activation of

the central nervous system, improved synchronization of motor units, and/or decreased input from inhibitory reflexes that limit strength in untrained subjects.

Hather et al. (1991) reported differences in the adaptive responses of the muscle fiber to the training regimens used by the subjects in the study by Dudley et al. (1991b). A significant ($P \leq 0.05$) increase in fiber area was promoted in both type II (32%) and type I (14%) muscle fibers only in the CON/ECC group after training. The double concentric group only showed an increase in type II fiber area (27%), while the CON group showed no significant increase in either fiber type. Consistent with the changes in strength reported by Dudley et al. (1991b) following 4 wk of detraining, the training-induced hypertrophy was only preserved in the CON/ECC group at this time. Hather et al. (1991) also reported a shift from type IIb to type IIa fibers after the 19 wk of training in all three groups. Only 0-1% of the fiber populations were type IIb after training. Similar fiber subtype transformations, although of smaller magnitude, have been reported following resistance training (Colliander and Tesch, 1990a; Staron et al., 1989). To more clearly verify the influence of this resistance training on muscle fiber composition, Adams et al. (1993) biochemically analyzed the original tissue samples for myosin heavy chain (MHC) composition. Their results showed that after training, IIb MHC composition decreased ($P \leq 0.05$) from 19% to

7%, while IIa MHC composition increased ($P \leq 0.05$) from 48% to 60%. These responses paralleled the alterations in fiber type percentages reported by Hather et al. (1991). Additionally, they support reports of relatively few type IIb but a higher proportion of type IIa fibers (Essén-Gustavsson and Tesch, 1990) and altered type II MHC isoforms (Klitgaard et al., 1990) in resistance trained athletes. Furthermore, it appears that the transformation in the type II subtype depends on the performance of concentric actions because all training groups in the Dudley et al. (1991b) study showed this response.

1.12.2 Muscle Damage, Growth Factors, and Satellite Cell Activation

A link has been established between muscle fiber damage, growth factor release, and satellite cell activation. Growth factors are simple peptides and are classified as hormones since they bind to specific cell surface receptors and can initiate a cascade of intracellular signalling events (White and Esser, 1989). One of these events is the stimulation of DNA replication through mitosis. Because adult muscle fibers are post-mitotic, the only source of new myofiber DNA is from satellite cell activation. Satellite cells can be activated and induced to migrate, proliferate and differentiate by a variety of stimuli including exercise, muscle damage, and mitogens (i.e., growth factors) (Bischoff, 1989; Darr and

Schultz, 1987; DeVol et al., 1990; Giddings et al., 1985; Sara and Hall, 1990; Schultz, 1989; Schultz et al., 1985; Summers et al., 1985; Vandenburgh, 1983, 1992; White and Esser, 1989; Yamada et al., 1989). Satellite cells are myogenic stem cells located beneath the basal lamina of mammalian skeletal muscle, and are assumed to participate in the repair and regeneration of damaged myofibers after exercise (Carlson and Faulkner, 1983). Following an injury, mononucleated satellite cells fuse to form a multinucleated myotube, which develops into a new muscle fiber to replace the damaged one (Schultz, 1989). Recruitment of satellite cells following injury appears to be confined to the damaged fiber (Schultz et al., 1986), suggesting that the regeneration process is aimed at simply synthesizing adequate contractile protein to repair the damaged fiber. However, the degree of their participation in the hypertrophy response (i.e., synthesis of additional contractile material) is not known and may depend on the type and/or extent of damage incurred by the muscle (White and Esser, 1989). The evidence that links the release of specific mitogens, caused by muscle damage, and their subsequent effect on satellite cell proliferation suggests that damage to contractile protein may in itself stimulate muscle growth. Nevertheless, further investigation of the mechanisms underlying muscle damage and growth factor/satellite cell

involvement in the hypertrophy process is essential before a conclusive link can be established.

1.13 SUMMARY

This review has presented information on the potential mechanisms regulating exercise-induced muscle hypertrophy. Specifically, muscle fiber injury, especially as the result of high specific tensions generated during eccentric muscle action, has been implicated as the signal linking the hypothesized threshold level of mechanical tension required to stimulate muscle growth. Repair and adaptation to these injured fibers, through synthesis of additional contractile material, may make muscle more resistant to future damage. Although the mechanisms contributing to this remain poorly understood, it has been clearly demonstrated that muscle hypertrophy is the result of increases in muscle protein synthesis.

The purpose of this investigation was to correlate the extent of myofibrillar disruption and the rate of protein synthesis in experienced resistance-trained subjects following an isolated bout of traditional concentric or eccentric exercise. The experiment was designed such that subjects concentrically lifted the weight with one arm and eccentrically lowered it with the other, ensuring that the same absolute work was performed by each arm. Subsequent

measures of muscle protein synthetic rate and ultrastructural configuration were initiated ~19 h post-exercise.

1.14 HYPOTHESIS

It was hypothesized that if, following resistance exercise, the process for repair of damaged fibers stimulates MPS then a correlation should exist between the extent of muscle damage and elevations in MPS.

CHAPTER II

CHANGES IN MUSCLE PROTEIN SYNTHETIC RATE AND ULTRASTRUCTURE FOLLOWING RESISTANCE EXERCISE

2.1 INTRODUCTION

Skeletal muscle hypertrophy, in response to a functional overload imposed by a program of resistance training, occurs when muscle protein turnover favours synthesis over degradation (Booth and Thomason, 1991; Smith and Rennie, 1990). The mechanical tension produced in the muscle with this type of training is thought to be the primary stimulus for growth (MacDougall, 1986; McDonagh and Davies, 1984; Vandenburgh, 1987), and exercised-induced muscle damage (Kuipers, 1994). Since it has been shown that both acute (Biolo et al., 1995; Chesley et al., 1992) and chronic (Rennie et al., 1980; Welle et al., 1995; Yarasheski et al., 1990a, 1993a, 1995) resistance training increases the muscle protein synthetic rate (MPS) in humans, it is possible that events associated with muscle damage and its repair may modulate MPS.

Changes in the magnitude and pattern of contractile activity modulate the expression of muscle-specific genes. Stimulation of MPS from isolated bouts of exercise seem to occur as a result of altered rates of translation (Barnett et

al., 1980; Bylund-Fellenius et al., 1984; Chesley et al., 1992; Goldspink, 1977; Goldspink et al., 1983; Laurent et al., 1978; Wong and Booth, 1990a, 1990b), while pretranslational and/or transcriptional control becomes increasingly important during chronic alterations in contractile activity (Brownson et al., 1988; Howard et al., 1989; Underwood and Williams, 1987; Williams et al., 1986; Wong and Booth, 1988, 1990b).

The rate of muscle protein synthesis following acute (Biolo et al., 1995; Chesley et al., 1992; MacDougall et al., 1995) and chronic (Rennie et al., 1980; Welle et al., 1995; Yarasheski et al., 1990a, 1992a, 1993a, 1993b, 1995) resistance exercise regimens has been examined using the reciprocal pool model of leucine metabolism. The primed-constant infusion of singly or doubly (L-[1-^{13}C] or L-[1,2-$^{13}C_2$]) labelled leucine allows the measurement of MPS from biopsy samples obtained several hours apart. Although this calculation is dependent on the enrichment of the true precursor for protein synthesis (leucyl-tRNA), plasma α-KIC enrichment has received experimental support as being a suitable surrogate index for this measurement (Smith and Rennie, 1990). Data from MacDougall and colleagues indicate, that in biceps brachii, MPS reaches a peak at approximately 24 h post-exercise (Chesley et al., 1992, MacDougall et al., 1995).

The cellular signals that alter exercise-induced gene transcription and translation are unknown; however, evidence suggests that exercise-induced growth factor/satellite cell activation may regulate MPS (Darr and Schultz, 1987; DeVol et al., 1990; Giddings et al., 1985; Schultz, 1989; Summers et al., 1985; Vandenburgh, 1992; White and Esser, 1989; Wong and Booth, 1990a, 1990b; Yamada et al., 1989). Satellite cell activation has been shown to be induced by a variety of stimuli including exercise resulting in muscle damage (Darr and Schultz, 1987; Gibala et al., 1995; Giddings et al., 1985; Vandenburgh, 1992; Schultz, 1989; White and Esser, 1989).

Heavy muscular exercise, especially activities involving an eccentric component, causes disruption of structural elements (i.e., Z disc and cytoskeletal proteins) of skeletal muscle, and its associated structures (i.e., sarcolemma, sarcoplasmic reticulum, basal lamina, mitochondria, extracellular matrix, surrounding connective tissue) (Fridén and Lieber, 1992; Stauber, 1989). The finding that eccentric muscle actions (EMA) cause more muscle damage than comparable concentric muscle actions (CMA) (Fridén et al., 1983a; Gibala et al., 1995; Newham et al., 1983a) has been attributed to physical tearing of sarcomeres (Fridén et al., 1981, 1983a) due to the greater tension generated per active fiber during EMA (Abbott et al., 1952; Asmussen, 1953; Bigland-Ritchie et al., 1976). The sequence, initiating factor(s), and time

course for events associated with exercise-induced muscle fiber injury are not known; however, the regenerative process is believed to be a normal precursor to muscle adaptation (Armstrong, 1984). This is supported by the finding that one bout of eccentric work protects against soreness and enzyme release during a subsequent bout (Balnave and Thompson, 1993; Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Nosaka et al., 1991), and that the muscles appear more resilient to future damage following regeneration (Byrnes et al., 1985; Clarkson et al., 1992; Fridén, 1984b). The observed increase in MPS following resistance exercise suggest that muscle damage may somehow stimulate an increase in MPS so that damaged fibers are repaired and made more resistant to further insults.

The purpose of this study was to correlate the extent of myofibrillar disruption and the rate of protein synthesis in experienced resistance-trained subjects following an isolated bout of traditional unilateral elbow flexor concentric or eccentric exercise. This was accomplished through electron microscopic analysis and the use of the reciprocal pool model of leucine metabolism of biopsy samples obtained from the concentrically or eccentrically exercised arm ~21 h post-exercise.

2.2 METHODOLOGY

2.2.1 Subjects

Six healthy males (age = 22.8 ± 1.1 yr, height = 175.8 ± 4.3 cm, mass = 78.8 ± 4.6 kg), who regularly participated in resistance training, served as subjects. All subjects were fully advised of the associated risks of the study and gave written informed consent. The study was approved by the University Ethics Committee (Appendix II).

2.2.2 Experimental Protocol

A) Preliminary Measures

Subjects performed all elbow flexor resistance exercises on an incline bench ("preacher curl bench") using a standard weight training dumbbell. One arm performed concentric muscle actions (CMA) only (concentrically-exercised (CON) arm), and the contralateral arm only performed eccentric muscle actions (EMA) (eccentrically-exercised (ECC) arm). Random assignment and counterbalancing of either CON or ECC arms was administered such that in 3 subjects the CON arm was their dominant arm, while in 3 subjects the CON arm was their non-dominant arm. Approximately seven days prior to the exercise bout each subject's one repetition maximum (1 RM) was determined for their CON arm. The criteria used to indicate that 1 RM strength was achieved were: 1) the ability to just

complete a strict repetition, and 2) the inability to perform a complete repetition with a heavier weight following a successful 1 RM attempt. Weights of the dumbbell were increased in multiples of 2 kg. Each subject's exercise bout weight was then established as 80% of their CON arm 1 RM.

B) Exercise Protocol

All subjects reported to the laboratory to perform the experimental protocol following 5 days of rest. The protocol that was used is as described elsewhere (Gibala et al., 1995). Subjects completed 8 sets of 6-10 repetitions, completing each action in 2.0 seconds. All sets were performed to muscular failure with 3 min of recovery between sets. A repetition was defined by the following order of events: (i) the subject began with the CON arm fully extended and the ECC arm fully flexed; (ii) the dumbbell was placed in the hand of the CON arm with the subject completing a CMA; (iii) upon completion, the dumbbell was removed and placed in the hand of the fully flexed ECC arm; (iv) the subject then completed an EMA by lowering the dumbbell to the fully extended position; (v) upon completion, the dumbbell was removed. In the event of a subject failing to complete a CMA the same absolute amount of work performed with each arm was controlled by performing the same number, or range of action, with the ECC arm.

C) Leucine Infusion

The labelled leucine infusion protocol began ~19 h after their exercise session, during which time subjects refrained from further activity. Two hours prior to the start of the infusion subjects consumed a standard meal consisting of ~1200 kcal of energy (~70% carbohydrate, 16% protein and 14% fat). The leucine infusion protocol that was used is similar to that described elsewhere (Chesley et al., 1992; MacDougall et al., 1995).

A 22 Ga plastic catheter was inserted into a suitable hand vein and an "arterialized" (Abumrad et al., 1981) blood sample (hot box at 65 ± 5°C) was obtained for the determination of baseline plasma [1,2-$^{13}C_2$]-alpha-ketoisocaproic acid (α-KIC) enrichment. This was used to approximate the true precursor enrichment for protein synthesis, leucyl-tRNA. A second catheter was inserted into a vein of the contralateral forearm for isotope infusion. A priming dose of L-[1,2-$^{13}C_2$]leucine (1 mg/kg) was administered followed by a constant infusion of L-[1,2-$^{13}C_2$]leucine (1 mg/kg/h) for approximately 10 h, delivered by a calibrated Harvard syringe pump. Arterialized blood samples, for the determination of [1,2-$^{13}C_2$]-α-KIC enrichment, were obtained prior to and at 30 min intervals over the last 2 h of the infusion. Blood was collected into heparinized tubes,

immediately centrifuged and the plasma stored at -20°C until analysis.

D) Muscle Biopsies

A total of 5 percutaneous needle biopsy samples were obtained from each subject under local anaesthesia. A 50 mL syringe was used to provide suction in order to assist in obtaining adequate sample sizes (Evans et al., 1982). One sample was obtained from the distal lateral portion of the biceps brachii of the non-dominant arm 3-5 weeks following the experimental protocol, and following 5 days where no training of the biceps brachii occurred, in order to establish baseline (BASE) indices of myofibrillar configuration. This sample was dissected free of fat and connective tissue and sectioned into smaller pieces and placed in a fixative (2% glutaraldehyde) for electron microscopy preparation.

The remaining 4 samples were obtained in a similar fashion from both the CON and ECC arm during the L-[1,2-$^{13}C_2$]leucine infusion protocol. The first set of biopsy samples were obtained 2 h following the leucine priming dose from both the CON and ECC arm. Two more biopsies (one from each arm) were taken at the end of the infusion. The samples were visibly dissected free of fat and connective tissue. During the first set of biopsies, small pieces of tissue from both

the CON and ECC arm were sectioned and placed in a fixative (2% glyceraldehyde) for electron microscopy preparation. The remaining samples were immediately frozen in liquid nitrogen and later transferred to a -70°C freezer. The 2 h period between the priming dose and the first set of biopsies was chosen to ensure that an isotopic plateau had been reached. This has been demonstrated in a prior study under similar conditions (Tarnopolsky et al., 1991).

2.2.3 Analytical Techniques

A) Electron Microscopy

Following initial fixation, the tissue was post-fixed in osmium tetroxide, dehydrated in graded baths of ethyl alcohol, and embedded in an epoxy resin (Spurr's). Four to eight blocks per specimen were obtained in this manner. Each block was semi-thin sectioned (0.5 μm) and stained with toluidine blue for light microscopy. An area containing at least 10 longitudinally oriented muscle fibers was identified, and ultra-thin sections (60-70 nm) were cut for electron microscopy. The sections were stained with uranyl acetate and lead citrate, mounted on 200 square copper grids, and viewed through a Philips 301 electron microscope (Philips Industries, Eindhoven, The Netherlands).

Longitudinal sections of fibers from each biopsy were analyzed for the presence of myofibrillar disruption. To be included in the analysis a fiber had to occupy at least 3 continuous square copper grids (area of 0.0217 mm^2). Based on this criterion, 40.9 ± 10.9 fibers (mean ± SD; range: 21-60) from each sample were examined in a blind fashion.

The classification scheme used in determining the extent of myofibrillar disruption of individual muscle fibers was as described by Gibala et al. (1995) shown in Table 1. Specifically, a fiber was considered disrupted if the myofibrillar architecture of any sarcomere appeared smeared. "Focal" damage was defined as an area of disruption occupying 1-2 adjacent myofibrils and/or 1-2 continuous sarcomeres (Newham et al., 1983a). An area of disruption occupying 3-10 adjacent myofibrils and/or 3-10 continuous sarcomeres was considered "moderate" damage. As well, a fiber which contained more than 10 focal areas of disruption was designated as "moderate" damage. "Extreme" damage was classified as an area of disruption spanning more than 10 adjacent myofibrils and 10 adjacent sarcomeres. Additionally, any fiber exhibiting more than 10 sites of moderate disruption was appointed an "extreme" rating of disruption. (Refer to Figures 1, 2, and 3 for sample photographs of each disruption category). The number of disrupted fibers (percent of total), and the

Table 1. Myofibrillar disruption classification scheme

Focal	Area encompassing 1-2 continuous sarcomeres and/or 1-2 adjacent myofibrils
Moderate	Area encompassing 3-10 continuous sarcomeres and/or 3-10 adjacent myofibrils, or more than 10 focal areas
Extreme	Area encompassing >10 continuous sarcomeres and >10 adjacent myofibrils, or more than 10 moderate areas

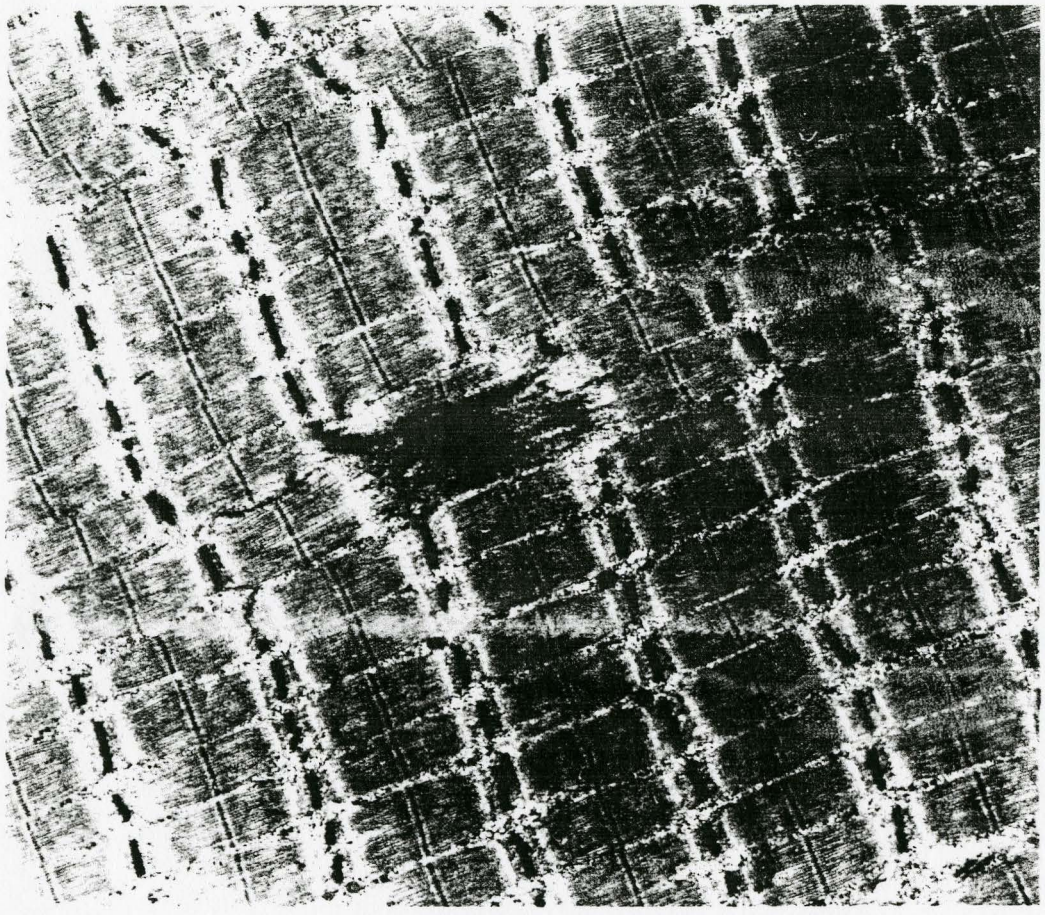


Figure 1. Micrograph illustrating focal area of disruption (x2600) (Gibala et al., 1995).

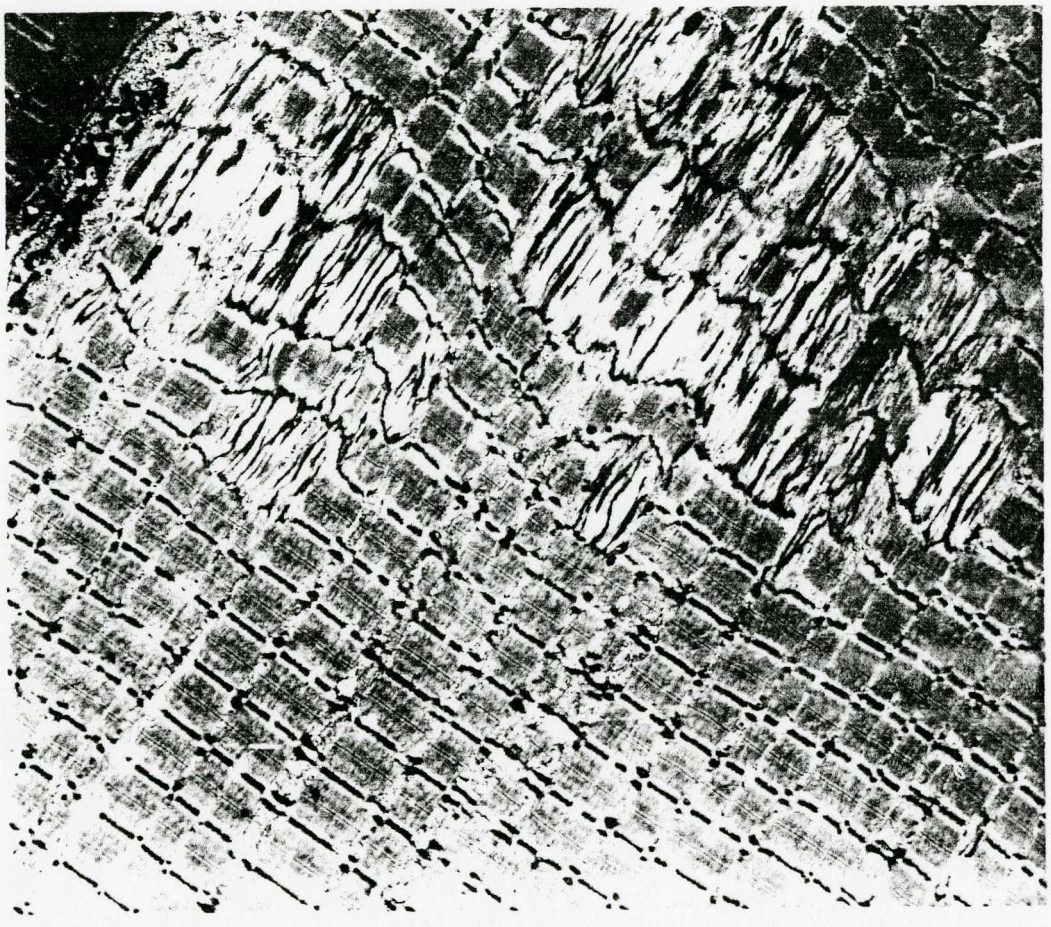


Figure 2. Micrograph illustrating moderate area of disruption (x720) (Gibala et al., 1995).

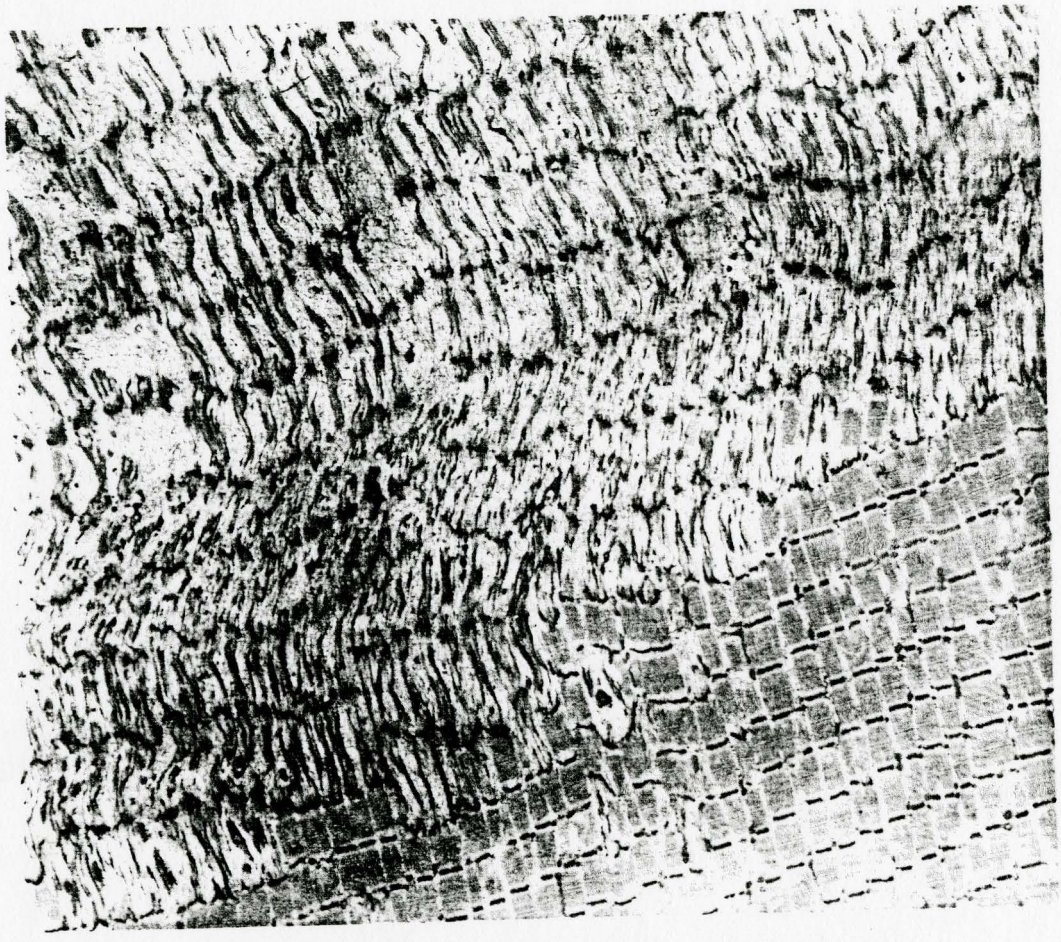


Figure 3. Micrograph illustrating extreme area of disruption (x550) (Gibala et al., 1995).

percentage of fibers in each disruption category (i.e. focal, moderate, extreme) were calculated and used for statistical analysis.

B) Muscle L-[1,2-$^{13}C_2$]leucine enrichment

Capillary gas chromatography-combustion-isotope ratio mass spectrometry was used to determine mixed muscle protein [1,2-$^{13}C_2$]leucine enrichment (see Yarasheski et al., 1992b). Briefly, 15-20 mg of frozen muscle tissue was powdered with a mortar and pestle chilled with liquid nitrogen. The muscle powder was homogenized in 1 mL 10% trichloroacetic acid and centrifuged (5000 rpm) to remove the supernatant. The pellet was washed (4x) with 1 ml normal saline, and hydrolyzed under nitrogen for 24-36 h with 2 ml of 6 N HCl. The hydrolysate was filtered (0.22 μm), then applied to an ion-exchange resin (Dowex AG-50W X8, 100-200 mesh, H^+ form) and washed (4-6x) with 1 ml 0.01 N HCl. The amino acids were eluted with 1.8 ml 6 M NH_4OH and the N-acetyl *n*-propyl ester derivative (NAP) prepared as described previously (Adams, 1974).

For analysis, 2 μl of derivatized sample was injected into a Hewlett-Packard 5980A gas chromatograph fitted with a 5% Phe Me capillary column (HP Ultra-2, 25m, 0.32 mm i.d., 0.52 μm film thickness) and operated in the splitless mode. The carrier gas was helium maintained at a column head

pressure of 10 psi. The injector and FID temperatures were 250°C and the oven temperature was programmed from 120°-240°C at 10°C/min, then 240°-300°C at 30°/min, where it was held for 3-5 min. As the NAP-leucine eluted from the column (7-8 min), it was directed away from the FID using a pneumatically controlled splitter union and carried (helium) through an oxidation furnace tube (800°C) packed with CuO granules, where complete combustion to CO_2 and water occurred. The water was subsequently removed in a trap (-100°C) and the CO_2 was carried by capillary line to a dual inlet isotope ratio mass spectrometer (SIRA II Series, VG Instruments, Danvers, MA) where the area under the ion current for masses 44 ($^{12}CO_2$), 45 ($^{13}CO_2$), and 46 were monitored and controlled by the manufacturer's computer software. Pulses (6 x 30s) of a calibrated reference CO_2 gas (-8 467 $\delta^{13}C$, -9 481 $\delta^{18}O$) were introduced into the mass spectrometer by a capillary line during each sample analysis before and after the CO_2 peak from the combusted NAP-leucine molecule eluted. The fittings on the system were routinely checked for leaks with argon gas (mass 40) and by monitoring the signal from ambient nitrogen gas (mass 28); these ion channels were always less than 10^{-12} A.

C) Plasma [1,2-$^{13}C_2$]-α-KIC Determination

The trimethylsilyl-quinoxalinol chemical derivative of plasma α-KIC was prepared as previously described (Schwarz et al., 1980). Electron ionization (EI) gas chromatography-mass spectrometry (GC/MS) was used to determine [$^{13}C_2$]-α-KIC enrichment by selective ion monitoring of mass/charge (m/e) 232 and 234. One microliter of derivatized sample was injected into a Hewlett-Packard 5980A gas chromatograph (MSD) fitted with a 50% cyanopropyl silicone capillary column (J&W Scientific DB-23, 30 m, 0.25mm i.d., 0.25 μm film thickness) with the injector operated in the split mode (11:1). The carrier gas was helium maintained at a column head pressure of 6 psi and flow adjusted to 0.8 mL/min. The injector temperature was 250°C, the column temperature was 170°C, and the retention time for α-KIC was 250 sec.

2.2.4 Calculations

The biceps muscle protein synthetic rate was calculated with the equation:

$$FMPS = (LEm \times 100)/K_{Ep} \times it)$$

where 'FMPS' is the fractional muscle protein synthetic rate (%/h), 'LEm' is the increment in L-[1,2-$^{13}C_2$]leucine abundance in muscle protein obtained between the two biopsies from each arm, 'K_{Ep}' is the mean plasma [1,2-$^{13}C_2$]-α-KIC enrichment for

t=8.0 8.5, 9.0, 9.5, and 10.0 h blood samples, and 'it' is the incorporation time (in hours) between biopsy samples taken from the same arm (Nair et al., 1988).

The leucine flux rate was calculated with the equation:

$$LF = (IR)(I_{tr}/I_{\alpha\text{-KIC}} - 1)$$

where 'LF' is the leucine flux rate (μmol/kg/h), 'IR' is the infusion rate, 'I_{tr}' is the tracer enrichment, and '$I_{\alpha\text{-KIC}}$' is the [1,2-$^{13}C_2$]-α-KIC enrichment.

2.2.5 Data Analysis

Electron microscopy data were analyzed using: (i) a one factor (1x3; arm) repeated measures ANOVA to examine the total number of fibers disrupted, and (ii) a two factor (3x3; arm x rating) repeated measures ANOVA to examine the severity of fiber disruption.

Leucine infusion parameters were analyzed using: (i) a one factor (1x5; sampling time) repeated measures ANOVA to examine plasma [1,2-$^{13}C_2$]-α-KIC enrichment, and (ii) a one factor (1x2; arm) repeated measures ANOVA to compare MPS between the CON and ECC arm.

A probability of $P \leq 0.05$ was representative of statistical significance. Significant main effects and interactions were further analyzed using a Tukey HSD post hoc test. Values are expressed as means ± standard error (SEM).

Correlational analysis (Pearson product) was used to determine a possible relationship between MPS and myofibrillar disruption. A correlational *t*-test ($P \leq 0.05$) was used to determine the significance of this relationship. Additionally, linear regression equations were created to predict MPS from indices of myofibrillar disruption.

2.3 RESULTS

2.3.1 Quantification of Myofibrillar Disruption

Quantification of myofibrillar disruption is summarized in Table 2. Specifically, 96.1% of the baseline (BASE) samples exhibited no fiber disruption. Four of the six BASE samples contained some evidence of disrupted fibers, but the average number of affected fibers was quite small: 2.9% focal and 0.9% moderate. Absolute disruption of fibers was significantly greater ($P = 0.007$) in the ECC samples (44.7 ± 11.4%) as compared to BASE (3.9 ± 2.0%). As well, ECC samples showed 40.27% greater total disruption than CON samples (44.7 ± 11.4% vs 26.7 ± 10.1%); however, this was not statistically significant.

A main effect for the extent of disruption (i.e., rating) appeared, when collapsed across samples. Both focal (11.23 ± 2.1%) and moderate (12.23 ± 4.1%) disruption was significantly ($P < 0.001$) greater than extreme disruption (1.60 ± 1.2%). A trend towards significance ($P = 0.059$) was evident in the

interaction of sample arm and rating. This revealed greater focal (19.62 ± 3.1%) and moderate (22.83 ± 8.3%) disruption within the ECC samples, compared to the same ratings within the CON (11.15 ± 1.8%, and 12.92 ± 3.1%) and BASE (2.93 ± 1.4%, and 0.93 ± 0.9%) samples. The total number of disrupted fibers, and number of disrupted fibers within each category, are illustrated in Figures 4 and 5, respectively.

2.3.2 Leucine Infusion Parameters

No significant difference was found between the MPS for the CON or ECC arm (0.0549 ± 0.0087 %/h vs. 0.0496 ± 0.0031 %/h, respectively). The mean plasma [1,2-$^{13}C_2$]-α-KIC enrichment did not differ across any of the sampling time points. These data are summarized in Table 3, and illustrated in Figures 6 and 7, respectively. The calculated leucine flux rate was 146.8 μmol/kg/h.

2.3.3 Correlation Between MPS and Fiber Disruption

A significant (P = 0.0196) positive correlation was found between ECC MPS and total number of ECC fibers disrupted ($y = 2.41 \times 10^{-4}x + 3.89 \times 10^{-2}$, $r = 0.886$, $r^2 = 0.7855$). No significant correlation existed between CON MPS and total number of CON fibers disrupted. These correlations are illustrated in Figure 8.

Table 2. Quantification of Disrupted Fibers

	FOC	MOD	EXT	TOT
BASE	2.9 ± 1.4	0.9 ± 0.9	0.0 ± 0.0	3.9 ± 2.0
CON	11.2 ± 1.8	12.9 ± 3.1	2.6 ± 2.0	26.7 ± 4.1
ECC	19.6 ± 3.1	22.8 ± 8.3	2.2 ± 1.5	44.7 ± 11.4[#]
X ± SEM	11.2 ± 2.1[*]	12.2 ± 4.1[*]	1.6 ± 1.2	25.1 ± 5.8

Number of disrupted fibers (percent of total; mean ± SEM) in each category. FOC = focal disruption, MOD = moderate disruption, EXT = extreme disruption, TOT = total number of disrupted fibers, BASE = baseline, CON = concentric, ECC = eccentric.

[*] $P < 0.001$ compared to EXT

[#] $P = 0.007$ compared to BASE

Table 3. Leucine Infusion Parameters

SUBJECT	INFUSION RATE (μmol/kg/h)	[$^{13}C_2$] αKIC (APE)	INFUSION TIME (h)	↑[$^{13}C_2$] LEU IN MUSCLE (APE)	PROTEIN SYN. (%/h)
1	7.25	5.22	10.52	CON 0.0201	0.0370
				ECC 0.0228	0.0420
2	7.98	4.46	9.15	CON 0.0372	0.0912
				ECC 0.0200	0.0491
3	7.89	4.52	9.33	CON 0.0240	0.0573
				ECC 0.0235	0.0530
4	7.60	3.84	10.02	CON 0.0251	0.0655
				ECC 0.0243	0.0627
5	7.45	6.28	10.07	CON 0.0222	0.0352
				ECC 0.0277	0.0485
6	7.34	4.84	10.03	CON 0.0212	0.0434
				ECC 0.0205	0.0426
X	7.58	4.86	9.85	CON 0.0250	0.0549
± SEM	0.12	0.34	0.21	0.0026	0.0087
				ECC 0.0231	0.0496
				0.0011	0.0031

CON = concentric arm, ECC = eccentric arm, APE = atom % excess

Figure 4. Number of disrupted fibers. (expressed as percent of total; mean ± SEM)

* P = 0.007 compared to BASELINE.

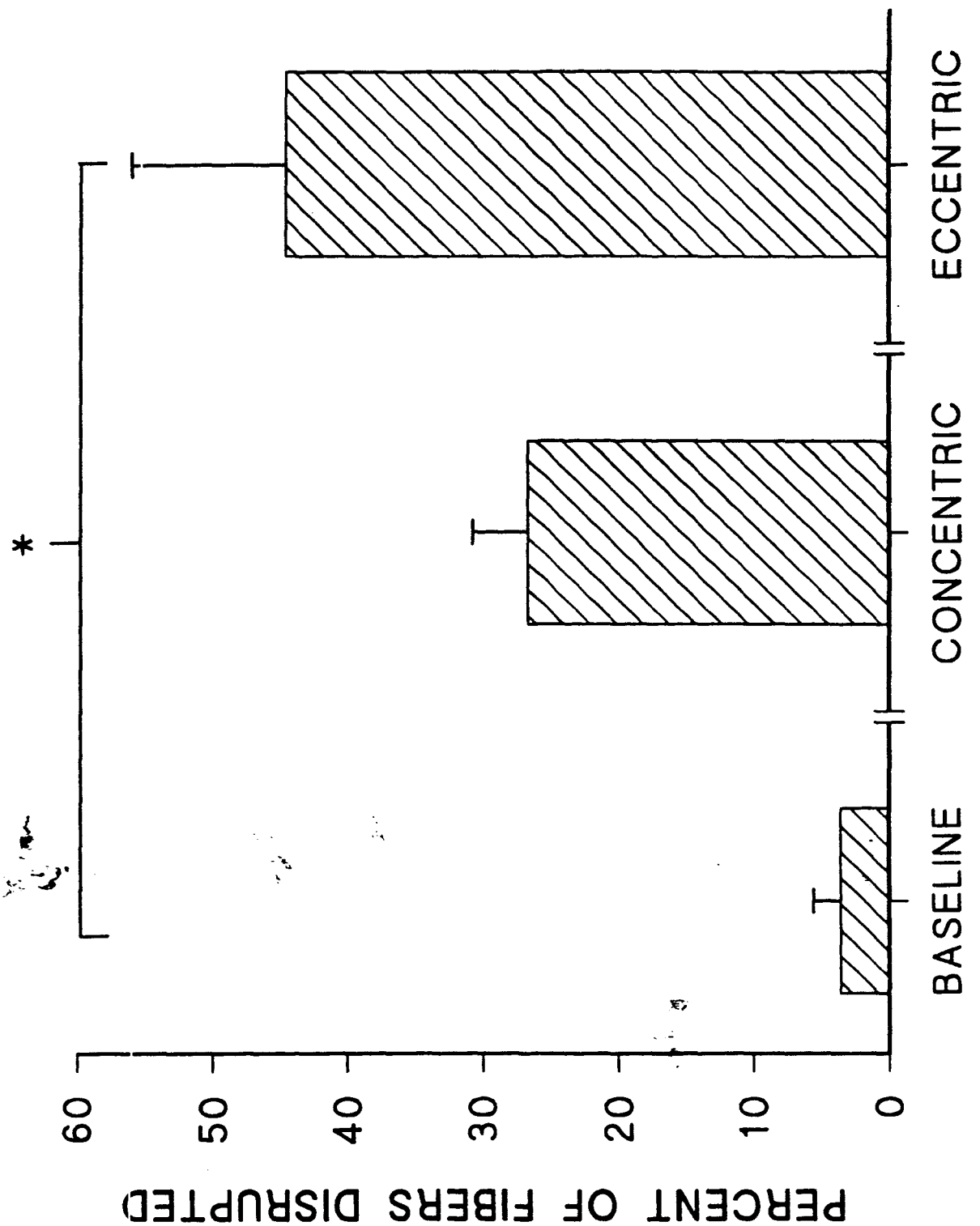

PERCENT OF FIBERS DISRUPTED
0
10
20
30
40
50
60
*
BASELINE
CONCENTRIC
ECCENTRIC

Figure 5. Number of disrupted fibers within each category. (expressed as percent of total; mean ± SEM)

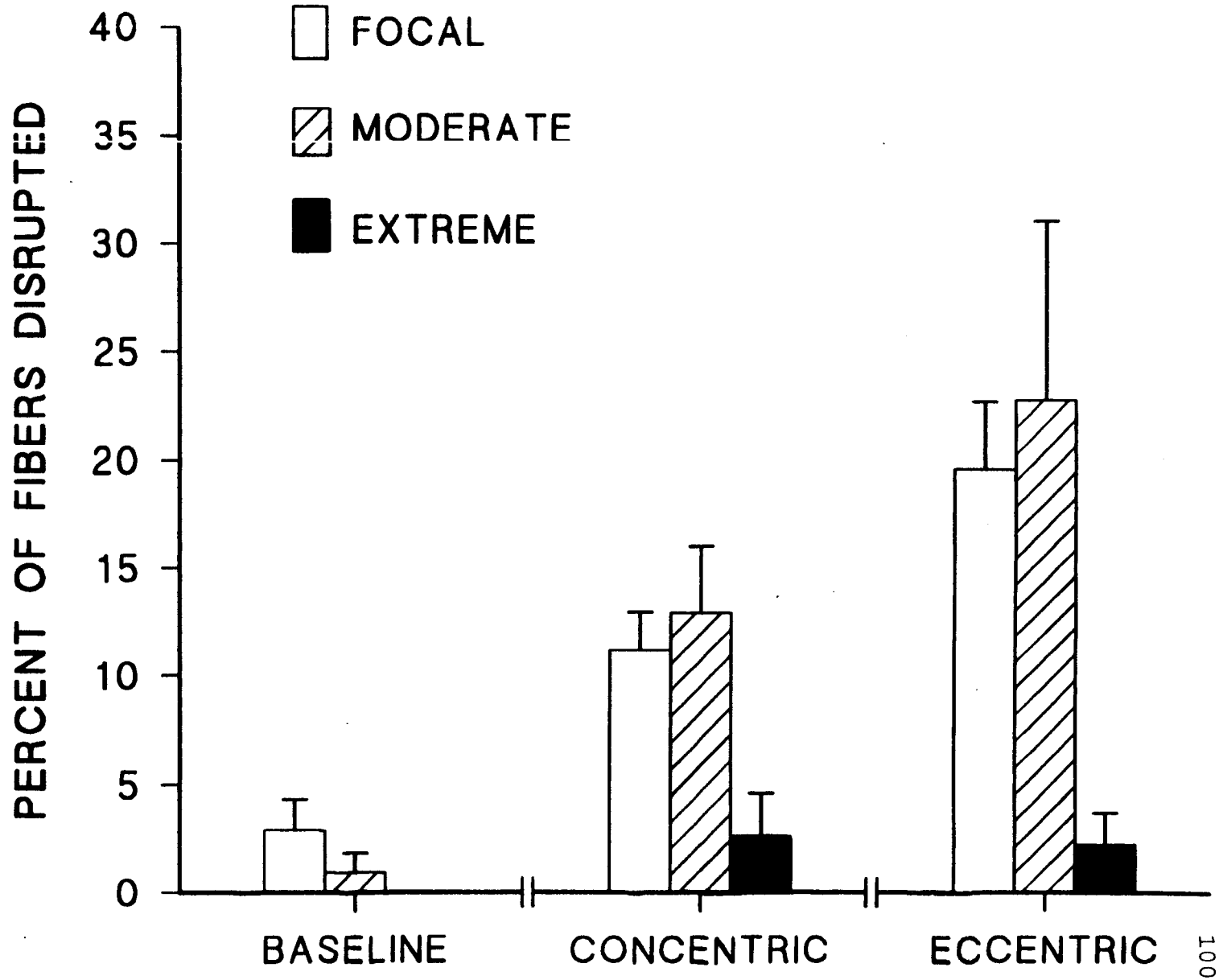

FOCAL
MODERATE
EXTREME
PERCENT OF FIBERS DISRUPTED
40
35
30
25
20
15
10
5
0
BASELINE
CONCENTRIC
ECCENTRIC
100

Figure 6. Biceps muscle protein synthetic rate in the CON versus ECC arm. (expressed as mean ± SEM)

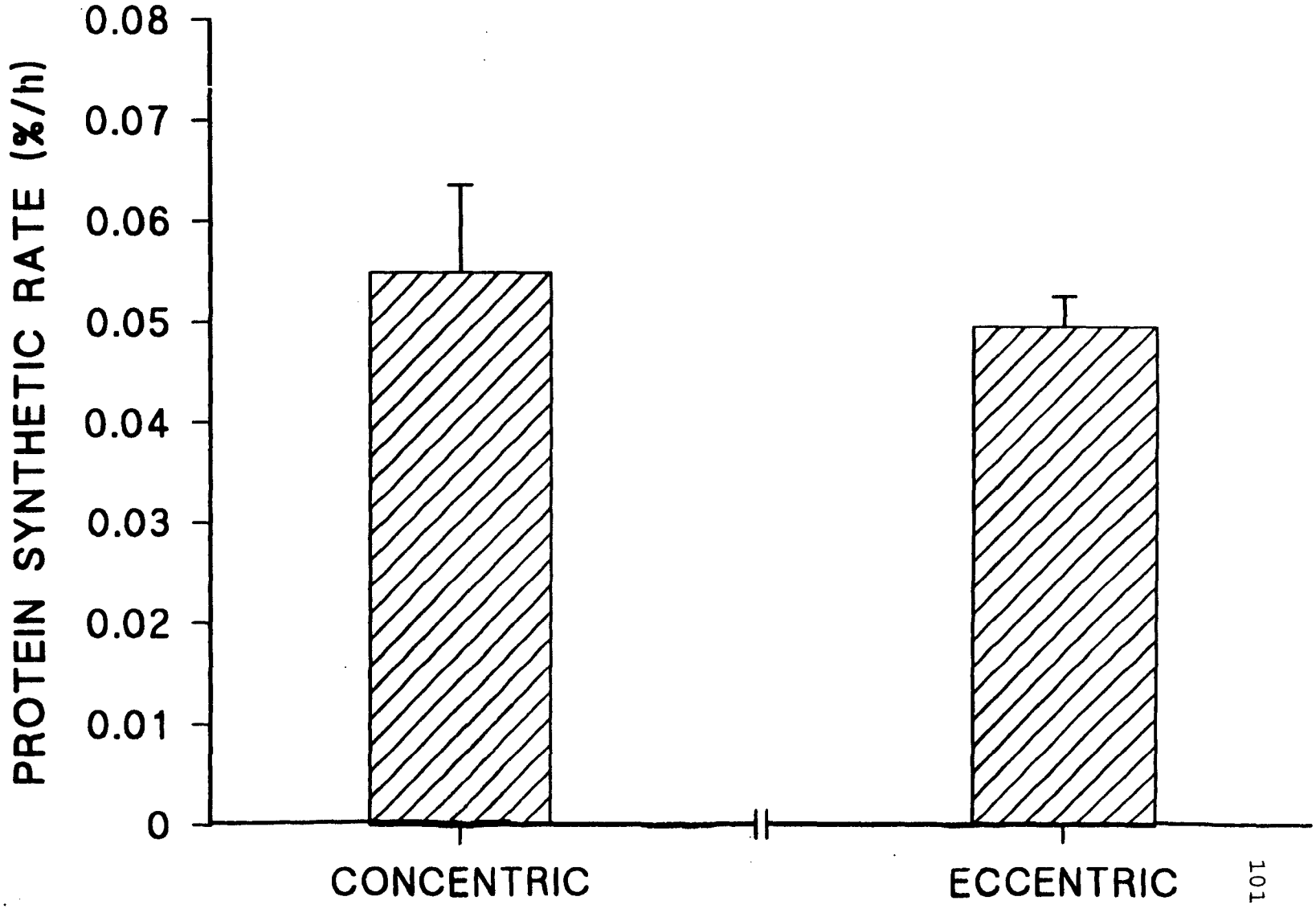

PROTEIN SYNTHETIC RATE (%/h)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
CONCENTRIC
ECCENTRIC

Figure 7. Enrichment of plasma [1,2-$^{13}C_2$]-α-ketoisocaproic acid over period during which muscle protein synthesis was estimated. (expressed as mean ± SEM)

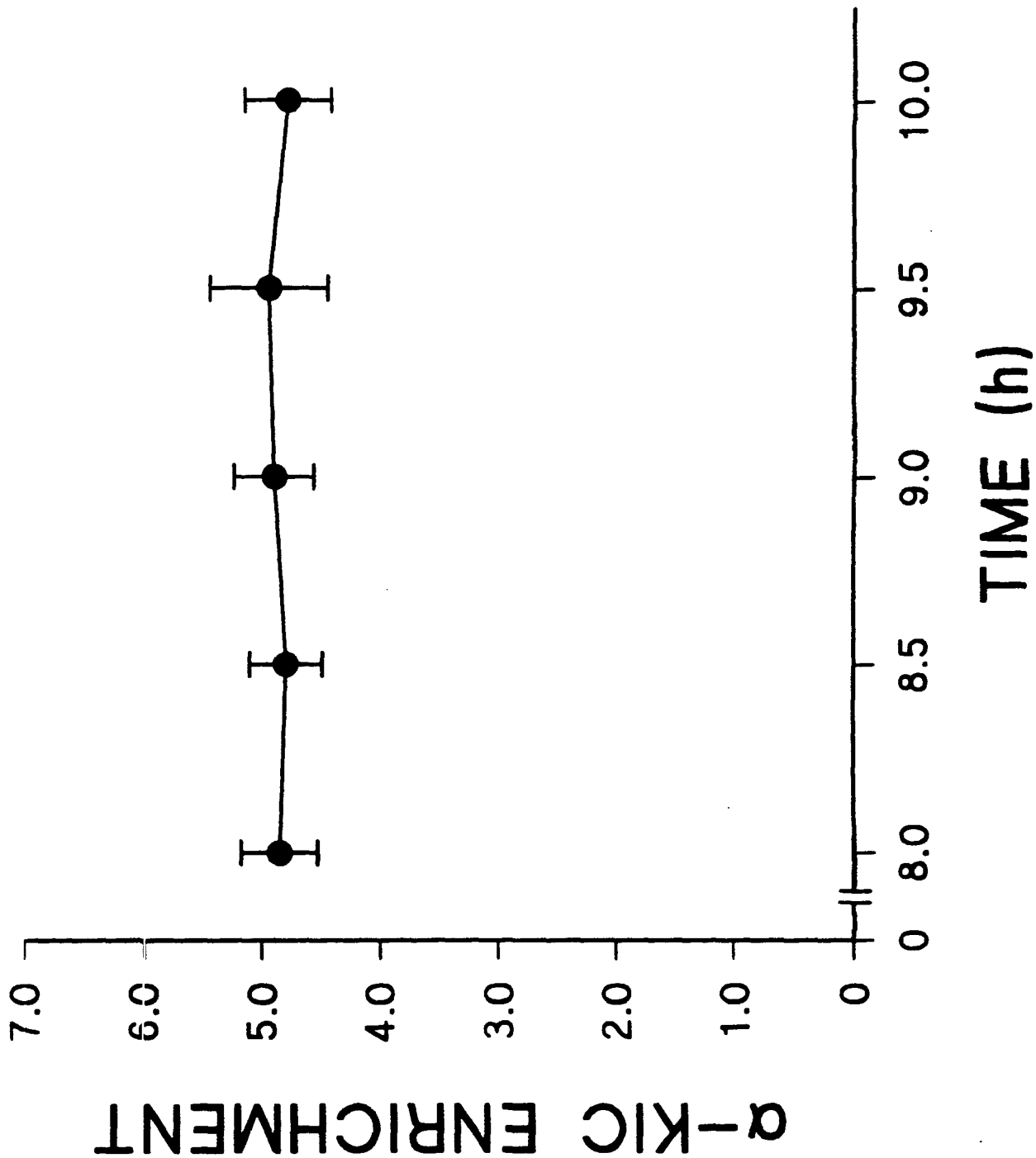
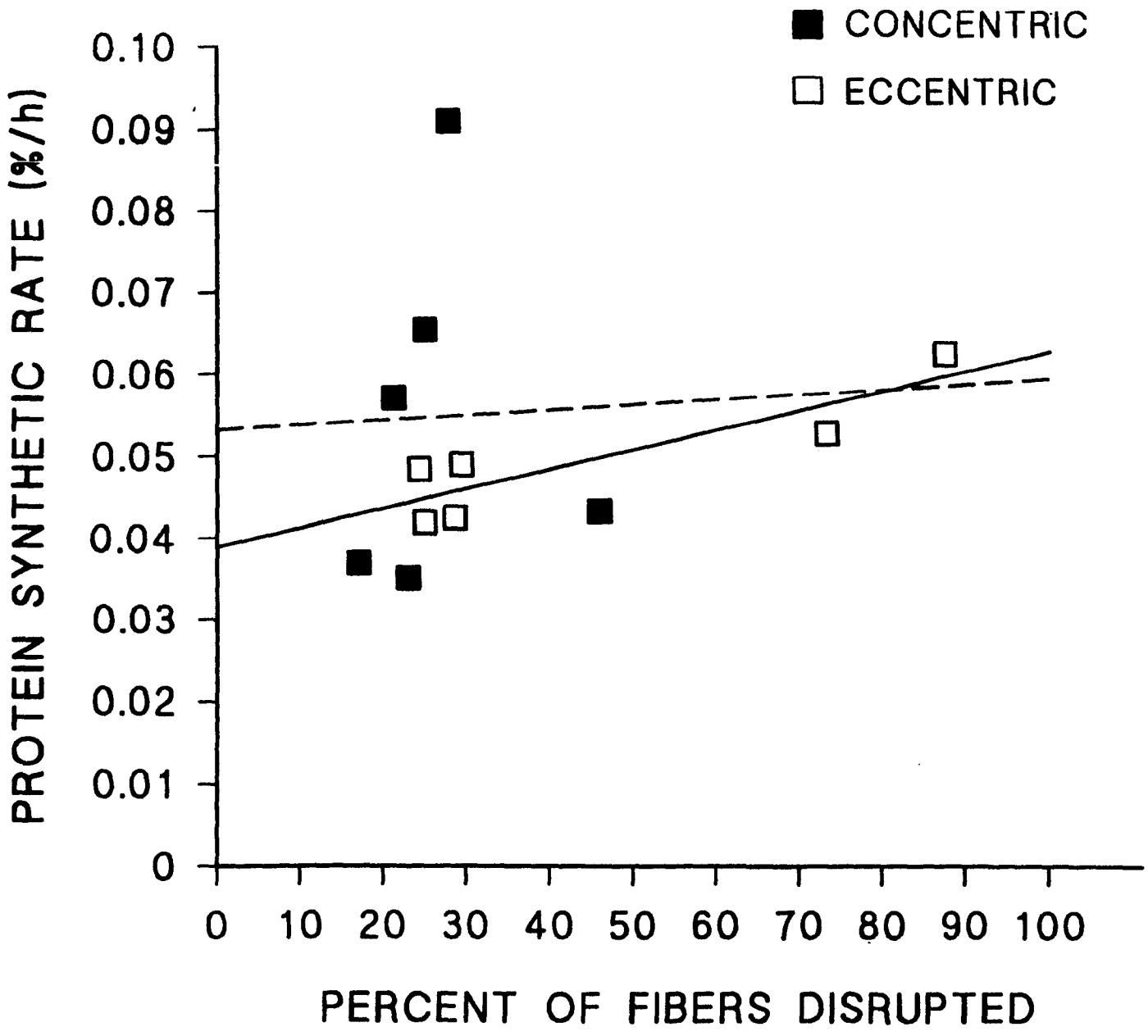

α-KIC ENRICHMENT
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
0
8.0
8.5
9.0
9.5
10.0
TIME (h)

Figure 8. Relationship between MPS and fiber disruption.

(ECC arm: $r = 0.886$ ($P = 0.0196$), $r^2 = 0.7855$)

(ECC arm: $y = 2.41 \times 10^{-4}x + 3.89 \times 10^{-2}$)

(CON arm: $y = 6.42 \times 10^{-5}x + 5.32 \times 10^{-2}$)


CONCENTRIC
ECCENTRIC
PROTEIN SYNTHETIC RATE (%/h)
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0 10 20 30 40 50 60 70 80 90 100
PERCENT OF FIBERS DISRUPTED

2.4 DISCUSSION

The methods used in this study to assess MPS and myofibrillar configuration relied respectively on the reciprocal pool model of leucine metabolism and electron microscopic analysis of biopsy samples. Although MPS reflects the contribution from soluble and myofibrillar proteins of the different fiber types, the most significant contribution (i.e., 70%) to total muscle protein comes from myofibrillar proteins (Smith and Rennie, 1990). Additionally, the use of plasma [1,2-$^{13}C_2$]-α-KIC enrichment as a valid index of the precursor pool available for protein synthesis has received experimental support (Smith and Rennie, 1990). Staron et al. (1992) and Gibala et al. (1995) have provided the only direct evidence of myofibrillar disruption following traditional (6-10 RM) heavy resistance training in humans. Earlier studies, using a variety of eccentrically-biased continuous exercise protocols (Fridén et al., 1981, 1983a, 1984; Newham et al., 1983a) demonstrated evidence of skeletal muscle disruptions at the light and electron microscopic levels. Since focal areas of injury may be difficult to detect using light microscopy, all analyses in the present investigation were conducted with an electron microscope in order to improve the resolution necessary for detecting small changes in fiber ultrastructure.

The major finding of this study was that, in trained subjects, an isolated bout of elbow flexor resistance exercise resulted in significant ($P \leq 0.05$) myofibrillar disruption in the eccentrically-exercised (ECC) arm but not the concentrically-exercised (CON) arm. In spite of this no difference was detected in MPS between the CON and ECC arms. This finding suggests that resistance exercise-induced muscle damage did not provide the activating signal for elevating MPS between ~21-29 h post-exercise. However, since myofibrillar disruption from a previous training session was essentially repaired within 5 d, MPS must have been elevated to repair the damage, either at a later time or chronically, within these 5 d.

The protocol used in the present study controlled the absolute muscle tension experienced by both arms by having the subjects lift the weight with one arm (concentric muscle action (CMA)), and lower the weight with the other (eccentric muscle action (EMA)). In spite of this, the ~40% greater total disruption identified in the ECC arm confirms previous observations (Gibala et al., 1995; Newham et al., 1983a) that EMA results in greater tissue disruption than comparable CMA. Studies using integrated electromyography (IEMG) have demonstrated fewer recruited motor units in the eccentrically, compared to the concentrically, acting limb (Bigland-Ritchie and Woods, 1976; Gibala et al., 1995; Newham et al., 1983b).

Therefore, the higher absolute tensions generated in the fewer active fibers during EMA may predispose them to greater injury.

Although the absolute amount of disruption in the CON arm was not significantly different from BASE, more than 25% of the fibers examined exhibited disruption. In contrast, Newham et al. (1983a) using a 20 min stepping exercise reported only morphological abnormalities in the eccentrically-exercised leg, and no evidence of fiber disruption in the concentrically-exercised leg. Myofibrillar disruption has, however, been reported in humans (Gibala et al., 1995) and cats (Giddings et al., 1985) following concentric weight-lifting exercise. As these authors concluded, we attribute the fiber disruption observed in the CON arm to the high tensions generated during the concentric phase of the movement. Ultrastructural examination showed random patterns of disruption across the fiber of both arms. In particular the myofibrillar cytoskeleton was believed to be affected, since the normal banding pattern or transverse alignment of sarcomeres were disrupted (Waterman-Storer, 1991). Two specific cytoskeletal intermediate filaments, desmin and titin, have been associated with eccentrically-biased exercise causing this type of disruption (Fridén et al., 1984; Fridén and Lieber, 1992) since they function in maintaining the lateral register of the sarcomere (Waterman-Storer, 1991).

The proportion of disrupted fibers from the ECC and CON arm was considerably lower (~50% and ~25%, respectively) and less severe than that in untrained subjects using the same protocol (Gibala et al., 1995). These findings substantiate the dynamic (Balnave and Thompson, 1993; Fridén et al., 1983b; Knuttgen et al., 1982) and high-force (Clarkson and Tremblay, 1988; Donnelly et al., 1992; Ebbeling and Clarkson, 1990; Nosaka et al., 1991) eccentric training studies which infer that the adaptive response to training makes the muscle more resistant to damage from subsequent bouts of similar exercise. Improved neural coordination (Sale, 1992) and reorganization of the contractile apparatus (Stauber, 1989) have been postulated as functional adaptations of the exercise trained muscles. Support for the latter hypothesis was provided by immunocytological signs of longitudinal extensions between successive type II fiber Z discs, attributed to sarcomerogenesis (Fridén et al., 1984). Waterman-Storer (1991) has suggested that following initial disruption of the intermediate cytoskeletal Z bridges, various cellular processes eventually prepare the damaged area for the insertion of new Z discs. Evidence of a non-lysosomal proteolytic pathway involving ubiquitin conjugated to abnormally folded or partially damaged proteins may be involved in these preparatory processes. Increased levels of conjugated ubiquitin have been observed in human biceps

brachii 48 h following high-force eccentric exercise (Reichsman et al., 1991; Thompson and Scordilis, 1994). Ubiquitin-mediated proteolysis of damaged myofibrillar proteins is reinforced by the 3-7 times higher density of conjugated ubiquitin found at the Z disc (the most often reported site of muscle damage; see Fridén et al., 1992) than anywhere else in the myofibril (Riley et al., 1988). The net effect of the structural alterations that occur results in a better distensibility of the fibers, reduced risk for mechanical damage and induces an optimal overlap between actin and myosin filaments (Fridén, 1984b).

The present study establishes that residual myofibrillar disruption from a previous training session is almost completely repaired within 5 d, since little or no disruption was apparent following 5 d without training. The low incidence of disrupted fibers (3.9%) at this point is similar to that occurring normally in healthy non-training humans (~2-7%) (Gibala et al., 1995; Meltzer et al., 1976), and probably reflects artifacts due to tissue handling and fixation. Using 70 maximal EMA (70-MAX) of the forearm flexors Clarkson and Tremblay (1988) and Ebbeling and Clarkson (1990) found that indirect indices of muscle damage (i.e., serum [CK], muscle soreness and pain questionnaires, isometric strength, and relaxed elbow joint angles) had not returned to baseline values by 5 d post-exercise in untrained subjects. More

importantly, subjects who performed 24 maximal EMA 2 wk prior to performing a subsequent bout of 70-MAX had restored these indices of muscle damage to baseline values by 3-4 d post-exercise (Clarkson and Tremblay, 1988). It therefore seems that muscular adaptations with eccentric or traditional resistance training attenuates the time course for recovery of exercise-induced muscle damage.

Measurements of MPS in biceps brachii at different time points following exercise indicate this measure to be significantly elevated by 50% and 109%, and non-significantly by 14% at approximately 4, 24, and 36 h post-exercise, respectively (Chesley et al., 1992; MacDougall et al., 1995). For this reason the 10 h labelled leucine infusion began ~19 h after the exercise session, so that the midpoint of the infusion would coincide with the peak MPS (i.e., 24 h). However, the exercise volume employed in these studies (4 unilateral sets of 6-12 RM for each of biceps curl, preacher curl, and concentration curl) was considerably greater than that of the present study (8 unilateral sets of 6-10 RM of either concentric or eccentric preacher curl). This is reflected in the differences in 24 h post-exercise MPS of Chesley et al. (1992) and that of the mean MPS for both CON and ECC arm of the present study (0.0944 vs. 0.0523 %/h, respectively). Indeed, it is possible that the exercise protocol used in the present study did not significantly

elevate biceps brachii MPS since comparisons with the MPS of control biceps brachii of experienced strength trained subjects at 4 h (0.067 %/h), 24 h (0.0452 %/h), and 36 h (0.0408 %/h) post-exercise are similar (Chesley et al., 1992; MacDougall et al., 1995). Numerous reports of a similar range (0.04-0.06 %/h) in vastus lateralis MPS in young untrained postabsorptive humans are available (Biolo et al., 1995; Carraro et al., 1990; Gibson et al., 1987, 1988; Griggs et al., 1989; Nair et al., 1988; Welle et al., 1995; Yarasheski et al., 1992a, 1993a). Evaluation of baseline biceps brachii MPS in our subjects was not possible due to the number of biopsies required for the other measurements.

In addition, the absence of difference between the CON and ECC MPS may have been a function of the relative efficiency in performing eccentric as compared to concentric muscle actions. If the critical stimulus for muscle protein synthesis or fiber hypertrophy, as hypothesized, is the amount of tension produced in a muscle (MacDougall, 1986; McDonagh and Davies, 1984; Vandenburgh, 1987), then equivalent MPS may have been expected since the CON and ECC muscles would have produced similar tension levels. However, as recently found by Mayhew et al. (1995), greater hypertrophy was exhibited in type II fibers of the concentrically acting vastus lateralis compared to the contralateral muscle exercising eccentrically at the same absolute force production (90% of the pre-exercise

maximal concentric power). Although total tension may not be the primary explanation for inducing a hypertrophic response, an increased relative use of the muscle may provide this stimulus (Goldberg et al., 1975). If this is true, the ECC arm in the present study would not have received as substantial a stimulus for protein synthesis since a greater efficiency has been associated with EMA. Although the exact mechanism is unknown this greater efficiency has been attributed to the connective tissue components and the visco-elastic properties of muscle undergoing active lengthening (Edman, 1992). If, however, one makes the assumption that it was the undamaged fibers that were responsible for protein synthesis than normalizing MPS with respect to the percentage of undamaged fibers revealed ~16.5% greater MPS in the ECC compared to CON arm (0.0897 vs. 0.0749 %/h, respectively).

The extent of myofibrillar damage, assessed at ~21 h post-exercise, was assumed to follow a similar time course as the peak in MPS (i.e., ~24 h post-exercise), since a similar study showed no difference in myofibrillar disruption either immediately or 48 h post-exercise (Gibala et al., 1995). These time points were therefore used in the present study to correlate the extent of myofibrillar disruption with elevations in MPS. The following reasons account for the employed timing and number of biopsies. A small section, from an initial biopsy taken at ~21 h post-exercise (used to

determine baseline enrichment of L-[1,2-$^{13}C_2$]leucine in the muscle for calculation of MPS) was used for ultrastructural configuration, circumventing the need for an additional biopsy at ~24 h post-exercise. Furthermore, Staron et al. (1992) have demonstrated unrepaired focal damage induced by the biopsy procedure for up to 2 weeks. Therefore any repeated biopsy sampling could have falsely increased the extent of exercise-induced damage. For this reason, baseline assessment of myofibrillar configuration occurred ≥3 wk following the experimental protocol.

The well known delayed onset muscle soreness (DOMS), experienced after unaccustomed exercise, consistently begins approximately 24 h following exercise, with the intensity peaking ~24-72 h post-exercise (see Miles and Clarkson, 1994). However, it is believed that DOMS follows a different time course and mechanism than indicators of muscle damage (Newham, 1988; Stauber et al., 1990). Fridén et al. (1983a) and Newham et al. (1983a) have reported progressive ultrastructural disruption in untrained subjects peaking ~30-72 h following 20-30 min of eccentric cycle ergometry and eccentric bench stepping. In contrast, untrained subjects performing heavy resistance concentric or eccentric arm curls showed no difference in the extent of fiber disruption from samples extracted immediately and 48 h post-exercise (Gibala et al.,

1995). This anomaly may be due to an underestimation in the actual number of focally disrupted fibers immediately post-exercise in the former two studies since light microscopy was used to quantify muscle damage. Alternatively, mechanical and metabolic factors, unique to longer duration, continuous eccentric exercise, may exacerbate initially damaged fibers. The higher specific tensions generated in type II fibers (Bodine et al., 1987), and their preferential involvement in eccentric cycle ergometry (O'Reilly et al., 1987) may make them more prone to mechanical damage. In addition, narrower Z discs (Eisenberg, 1983), and an inability to regenerate ATP once fatigued (placing these fibers in a high-stiffness state), could lead to mechanical disruption from a subsequent stretch (Fridén and Lieber, 1992). Beginning shortly after, and continuing for several days, exercise-induced muscle damage activates several degradative factors intrinsic to the fiber (i.e., Ca^{2+}-activated neutral proteases, lysosomal proteases, phospholipases, and the cascade of degradative reactions initiated by these enzymes) (Armstrong, 1990) as well as phagocytic processes (Armstrong et al. 1991) that ultimately lead to, or result from, the loss of cellular Ca^{2+} homeostasis. These degradative processes, along with the previously mentioned proteolysis attributed to conjugated ubiquitin, presumably account for the observed progressive

nature of myofibrillar disruption. It is noteworthy that Z band streaming, which has been attributed to degradation of the Z disc components by Ca^{2+}-activated neutral proteases (Busch et al., 1972; Ishiura et al., 1980), was not observed in either this study or the Gibala et al. (1995) study.

Though it seems considerable disruption may still exist ~36 h post-exercise, evidence suggests that biceps brachii MPS has returned to basal values (MacDougall et al., 1995). Armstrong et al. (1991) proposed that the regenerative phase, which restores the muscle to its normal or improved condition, begins 4-5 d following muscle injury. This restoration, and the possibility of synthesizing additional contractile material presumably occurs earlier in experienced athletes (Clarkson and Tremblay, 1988), but may go undetected by the measurement technique. Indeed the apparent absence of an elevated MPS consequent to performing this exercise protocol seems a larger stimulus than the subsequent repair of damaged myofibrils. It should be noted that the measured rate of muscle protein synthesis reflects overall synthesis and not a net rate of muscle growth, since it does not account for the simultaneously occurring muscle degradation. Urinary 3-methylhistidine (MH) excretion, an index of myofibrillar protein degradation, has been shown to be chronically elevated in response to a resistance training program (Pivarnick et

al., 1989). However, several major assumptions concerning the method have led investigators to conclude that 3-MH production should be used only as a semi-quantitative index of whole-body actin and myosin turnover (Smith and Rennie, 1990). In spite of the lack of a valid technique to assess the fractional rate of protein degradation in muscle, evidence from longitudinal resistance training studies (Cureton et al., 1988; Davies et al., 1988; Frontera et al., 1988; Housh et al., 1992; Ikai and Fukunaga, 1970; MacDougall et al., 1977; Narici et al., 1989; Young et al., 1983) infer that concurrent increases in the rate of muscle protein synthesis and degradation occur, otherwise the reported 5-34% increase in muscle cross-sectional areas would have been much greater.

In addition, the present study indirectly demonstrated that concurrent catabolic and anabolic processes occur, since virtually complete restoration of residual myofibrillar disruption from a previous training session was achieved within 5 d. Degradative processes, initiated and regulated by phagocytic cells, probably dominate the progression of necrosis by 2-6 h, and continue for several days after the injury (Armstrong et al., 1991). Also, the elevated levels of conjugated ubiquitin observed 48 h following muscle damaging exercise (Reichsman et al., 1991; Thompson and Scordilis, 1994), suggests that initial elevations in degradation either precede and/or are eventually overtaken by an enhanced MPS in

order to restore the muscle to its normal or improved condition. Alternatively, damaged myofibrils may simply be scavenged, with subsequent compensatory hypertrophy or hyperplasia of myofibrils. However, this possibility does not agree with implications of an upper limit for cross-sectional fiber and myofibril areas (Goldspink, 1992), or the lack of evidence concerning hyperplasia in humans (MacDougall, 1992).

Collectively, the previous discussion implies that muscle damage induces an enhanced degradative response which is at least matched by an increase in MPS in an attempt to repair damaged myofibrils. The obtained correlation between individual values for MPS and the % of disrupted fibers in tissue from the ECC arm may reflect inter-individual differences in the relative severity of the exercise. Alternatively, two separate mechanisms may have existed in regulating MPS. The greater relative use of biceps brachii in the CON arm may have affected CON MPS to a similar degree as the greater myofibrillar disruption affected ECC MPS. This would explain the similar MPS between arms. The finding that contractile forces of greater than ~66% of 1RM are necessary for hypertrophy and strength gains (McDonagh and Davies, 1984) may support this suggestion. Because both arms lifted a weight equivalent to 80% of the subject's concentric 1RM, and since the forces that can be generated in a CMA are ~50% less than an EMA (Edman, 1992), the stimulus threshold may not have been

reached in the ECC arm. However, the ~40% greater disruption in the ECC than CON arm seems reasonable for providing a repair stimulus to affect ECC MPS.

2.4 CONCLUSIONS

The major finding of this study was that, in trained subjects, an isolated bout of elbow flexor resistance exercise resulted in significant myofibrillar disruption in the eccentrically-exercised but not the concentrically-exercised arm. In spite of this no difference was detected in MPS between the concentric and eccentric arms. This finding suggests that resistance exercise-induced muscle damage did not provide the activating signal for elevating MPS between 21-29 hours post-exercise. However, since myofibrillar disruption from a previous training session was essentially repaired within 5 days, net MPS must have been elevated to repair the damage, either at a later time and/or chronically, within these 5 days.

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APPENDIX I: ANOVA SUMMARY TABLES

APPENDIX I-A
ANOVA SUMMARY FOR OVERALL PERCENTAGE OF DISRUPTED FIBERS

SOURCE	SS	DF	MS	F	P
BLOCKS/SUBJECTS	1559.540	5			
ARM	5085.423	2	2542.712	8.465	.007
ERROR	3003.657	10	300.366		
TOTAL	9648.620	17			

BAB	CON	ECC	
5	6	6	
3.58	26.67	44.65	24.97
4.93	10.09	28.05	
121.29	508.83	3933.07	

APPENDIX I-B
ANOVA SUMMARY FOR PERCENTAGE OF DISRUPTED FIBERS WITHIN EACH CATEGORY

SOURCE	SS	DF	MS	F	P
BLOCKS/SUBJECTS	527.481	5			
ARM	1671.014	2	835.507	8.437	.007
ERROR	990.326	10	99.033		
RATING	1240.438	2	620.219	20.430	<.001
ERROR	303.582	10	30.358		
ARM RATING	630.671	4	157.668	2.704	.059
ERROR	1166.303	20	58.315		
TOTAL	6529.814	53			
(RESIDUAL)	2460.211	40			

	FOC	MOD	EXT	
	6	6	6	
	2.93	.93	0.00	
BAS				1.29
	3.33	2.29	0.000	
	55.51	26.13	0.000	
	6	6	6	
	11.15	12.92	2.60	
CON				8.89
	4.37	7.59	5.01	
	95.43	287.69	125.30	
	6	6	6	
	19.62	22.83	2.20	
ECC				14.88
	7.63	20.21	3.57	
	290.95	2042.81	63.86	
	11.23	12.23	1.60	

APPENDIX I-C

ANOVA SUMMARY FOR MPS RATE

SOURCE	SS	DF	MS	F	P
BLOCKS/SUBJECTS	16.510	5			
ARM	.837	1	.837	.456	
ERROR	9.169	5	1.834		
TOTAL	26.517	11			

CON	ECC	
6	6	
5.49	4.97	5.23
2.13	.76	
22.77	2.91	

APPENDIX I-D

ANOVA SUMMARY FOR [1,2-$^{13}C_2$]-α-KIC ENRICHMENT

SOURCE	SS	DF	MS	F	P
BLOCKS/SUBJECTS	17.308	5			
TIME	.112	4	.028	.147	
ERROR	3.809	20	.190		
TOTAL	21.229	29			

1	2	3	4	5	
6	6	6	6	6	
4.85	4.80	4.90	4.95	4.79	4.86
.80	.76	.83	1.23	.90	
3.20	2.87	3.48	7.52	4.04	

APPENDIX I-E

CORRELATION AND REGRESSION SUMMARY FOR MYOFIBRILLAR DISRUPTION AND MPS

OVERALL MEANS AND DISPERSIONS

VARIABLE	MEAN	VARIANCE	STAND.DEV.	STAND.ERR.
1 MPS C	5.493333E-02	4.553107E-04	2.133801E-02	8.711206E-03
2 MPS E	.04965	5.827502E-05	7.633808E-03	3.116489E-03
3 DAM C	26.66667	101.7667	10.08795	4.118387
4 DAM E	44.65	786.6151	28.04666	11.45

VALUES FOR r

	DAM E	DAM C	MPS E
MPS C	+.303	+.030	+.451
MPS E	+.886	-.257	
DAM C	-.205		

VALUES FOR r-SQUARED

	DAM E	DAM C	MPS E
MPS C	.0915	.0009	.2031
MPS E	.7855	.0659	
DAM C	.0422		

Values for t-test on Pearson r
4 degrees of freedom

	DAM E	DAM C	MPS E
MPS C	0.635	0.061	1.010
MPS E	3.828	0.531	
DAM C	0.420		

2-TAILED PROBABILITY VALUES
(Half this value is the 1-tailed probability)

	DAM E	DAM C	MPS E
MPS C	.5631	.9092	.3716
MPS E	.0196	.6251	
DAM C	.6941		

REGRESSION FORMULAS & PREDICTIONS

Y'=a + bX

a= .0532224

b= 6.415976E-05

Predicted MPS C= .0532224 + 6.415976E-05 (DAM C)

a= 3.887886E-02

b= 2.412349E-04

Predicted MPS E= 3.887886E-02 + 2.412349E-04 (DAM E)

FULL CORRELATION MATRIX

	MPS C	MPS E	DAM C	DAM E
MPS C	+1.0000	+0.4507	+0.0303	+0.3025
MPS E	+0.4507	+1.0000	-0.2568	+0.8863
DAM C	+0.0303	-0.2568	+1.0000	-0.2055
DAM E	+0.3025	+0.8863	-0.2055	+1.0000

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APPENDIX II: SAMPLE CONSENT FORM

"THE EFFECTS OF CONCENTRIC AND ECCENTRIC RESISTANCE EXERCISE ON MUSCLE ULTRASTRUCTURE AND PROTEIN SYNTHETIC RATE"

INFORMATION AND CONSENT FORM

The principal investigators for this project are Dr. Duncan MacDougall and Stephen Interisano. They will provide you with a detailed verbal description of the procedures involved in the study. In addition, you are asked to carefully read the following information form and sign it if you wish to be a subjects for this study.

A. PURPOSE

The purpose of this study will be to examine the effects of a single session of concentric and eccentric resistance exercise on muscle ultrastructure and protein synthetic rate.

B. PROCEDURES

During your first visit to the lab your maximal elbow flexor strength will be assessed. During your second visit to the lab (approximately one week later, and after not having trained your elbow flexors for at least 5 days), you will be asked to perform an arm curl exercise with each arm separately. One arm will perform only concentric muscle actions (i.e., lifting the weight), while the other arm will only perform eccentric muscle actions (i.e., lowering the weight). Approximately 80 repetitions in total will be performed with each arm. Approximately 17 hours after this exercise session you will report to the lab to consume a standard diet. Two hours later you will receive an injection of a small amount of an amino acid (leucine) into a vein in your arm. The only difference between this substance and that which you would normally consume in your daily food is that it has been labelled with a non-radioactive tracer so that it can be identified as being different than the protein which is naturally found in your muscle. This procedure will take approximately 10 hours, and will require an overnight stay in the lab.

During this time, two samples of muscle will be taken from the biceps muscle of each arm (four samples in total) by way of a needle biopsy procedure. This

...2

technique involves the removal of a small amount of tissue (under local anaesthesia) by a skilled physician using a special needle. From these samples, the amount of labelled leucine that is taken up into muscle protein can be determined and a protein synthetic rate can be calculated. In addition, pieces of these samples will be analyzed for various indices of muscle damage. By comparing the concentrically and eccentrically trained arms, it can be determined how effective the intervening exercise bout was in stimulating muscle damage, amino acid uptake and muscle protein synthesis. The biopsy samples will be taken from each arm approximately 2 hours following the start of the tracer infusion, and again at the end of the infusion protocol. In addition, blood samples will be drawn from a hand/arm vein periodically throughout the infusion protocol.

The final visit to the lab will take place approximately 3-4 weeks later, at which time another biopsy will be taken from one arm in order to determine a "baseline" index of muscle ultrastructure. You will again refrain from training your elbow flexors for at least 5 days prior to this visit.

C. DETAILS OF THE PROCEDURES AND THEIR POSSIBLE RISKS

(1) Labelled Leucine Infusion

Leucine is an amino acid which you consume daily in your food when you eat meat or dairy products. It is an essential amino acid, which means that your body cannot manufacture it on its own, and thus it is ideal for tracer studies. A small tube (catheter) will be placed into a vein in your arm and sterile non-radioactive labelled leucine will be infused into your vein over a period of 10 hours using a Harvard infusion pump. There is no discomfort associated with this technique, and it is expected you will sleep through most of the procedure.

The entire procedure will be carried out by a skilled physician with strict adherence to sterile procedures. Following the infusion, there may be slight bruising around the needle area which may persist for up to three days. Since the injected tracer is not radioactive, there is no known risks to health/fertility associated with this procedure (which has been used in studies involving human subjects for over 30 years).

(2) Needle Biopsy Procedure

This procedure involves the local injection of an anaesthetic ("freezing") into the skin of the biceps area, after which a small (4 mm) incision will be made and a small (50-100 mg) piece of muscle will be removed using a sterile hollow needle. After the procedure, a suture will be used to close the skin, and ice and pressure will be applied to minimize bruising. The procedure will be performed by a skilled physician who is thoroughly familiar with the needle biopsy technique. ...3

Complications with the procedure are rare. However, in our experience with athletes, fewer than 1 in 400-500 subjects experience local skin infection, 1 in 30-40 have a temporary (up to four months) localized loss of sensation in the skin at the site of the incision, and a few subjects have mild bruising around the incision for 4-5 days. There is also the very rare (one in a million) chance that you may be allergic to the local anaesthetic.

D. CONFIDENTIALITY OF RESULTS

The data collected will be used in preparation of reports to be published in scientific journals. Subjects will not be identified by name in these reports. You will have access to your own data and the group data when it is available for your own interest.

E. REMUNERATION

You will receive an honorarium of $150.00 for the completion of the study to help compensate for your time commitment.

F. FREEDOM TO WITHDRAW FROM THE STUDY

You are free to withdraw from the study at any time. If, after reading the above information, you are interested in participating as a subject you should read the statement below and sign the space provided.

I HAVE READ AND UNDERSTAND THE ABOVE EXPLANATION OF THE PURPOSE AND PROCEDURES OF THE PROJECT AND AGREE TO PARTICIPATE AS A SUBJECT.

Signature: _____

Date: _____

Witness: _____