MUSCLE PROTEIN SYNTHESIS FOLLOWING RESISTANCE EXERCISE

CHANGES IN MUSCLE PROTEIN SYNTHESIS FOLLOWING RESISTANCE EXERCISE

By

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ABSTRACT

In order to gain better insight into the possible mechanisms that influence resistance training-induced muscle hypertrophy, two groups of subjects were examined for changes in muscle protein synthesis, and protein, total RNA, and DNA content 4 (group A) and 24 (group B) hours following an isolated bout of unilateral elbow flexor resistance training. Subjects trained one arm by performing 3 different biceps exercises consisting of 4 sets of 6-12 repetitions to failure while the contralateral arm served as a control. Both groups received a primed-constant infusion of L-[1-^{13}C] leucine (group A infused 0.68h post-exercise for 5.4h;group B infused 20.41h post-exercise for 6.38h) and muscle protein synthesis was determined by the increment in L-[1-^{13}C] leucine abundance in muscle biopsy samples relative to the mean plasma α-KIC enrichment at isotopic plateau. Protein, total RNA, and DNA were determined with standard methods and RNA capacity (total RNA (ug)/protein (ug)) and RNA activity (ug protein synthesized/hour/ug RNA) were calculated to assess changes in gene transcription and translation. Possible muscle damage was assessed by changes between pre and 15 minute post-exercise maximal voluntary elbow flexor torque (measured at 30°/s and 180°/s) and by 22 hour post-exercise changes in serum CK

activity (assessed in group B only).

Both groups had significantly elevated muscle protein synthetic rates (group A 43% ↑; group B 80% ↑) and RNA activities (group A 25% ↑ ; group B 89% ↑) in the exercised biceps compared to the control biceps. In addition, post-exercise torque declined by 22% at 30°/s and by 24% at 180°/s and mean serum CK activity increased by 35% in group B. It is concluded that an intense bout of resistance training stimulates increases in muscle protein synthesis 4 and 24 hours post-exercise. The elevations in muscle protein synthesis of the exercised arm are probably related to increases in translation with contractile protein damage being one possible signal for increasing protein synthetic rates.

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. Chapter II embodies 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: INDUCTION, MECHANISMS, AND METHODOLOGY

1.1 INTRODUCTION

Skeletal muscle possesses a remarkable ability to adapt to increased functional demands. This muscle plasticity is manifested by both acute and chronic changes in the content of specific muscle proteins and is related to the intensity, duration, frequency and pattern of contractile activity (Booth et al.,1982; Saltin & Gollnick,1983). Work-induced muscle hypertrophy represents an adaptation to a specific stimulus and is one example of muscle plasticity. The study of muscle hypertrophy, following a program of resistance training (weight training), has received considerably less attention than physiological adaptations following aerobic activity.

Skeletal muscle hypertrophy may be defined as the growth of existing muscle fibers by increases in myofibril cross-sectional area and myofibrillar proliferation (Ashmore and Summers,1980; MacDougall,1986). Myofibrillar growth and proliferation occur only when protein synthetic rates exceed protein degradation rates (Waterlow et al.,1978). Information about human skeletal muscle hypertrophy has been obtained

largely from longitudinal weightlifting/bodybuilding studies and cross-sectional comparisons between elite strength-trained athletes and sedentary control subjects. A summary of these investigations indicates that a program of heavy resistance training can lead to: 1) large increases in muscle strength due to neural adaptations and hypertrophy of type I and type II muscle fibers, 2) a decrease in mitochondrial volume density, 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; MacDougall et al., 1979; MacDougall, 1986; Sale,1988; Tesch et al.,1984; Tesch,1987). Changes in the activities of key enzymes of the glycolytic, tricarboxylic acid, and B-oxidation pathways are variable and depend on the magnitude of the hypertrophy response and the type of resistance training performed (Dudley,1988; Tesch,1987; Tesch et al.,1989).

Whether or not muscle hypertrophy in adult humans is also the result of an increase in fiber number is controversial. Muscle fiber hyperplasia has been documented in several animal species in response to experimental protocols that induce muscle hypertrophy (Alway et al.,1989; Gonyea et al.,1986; Ho et al.,1980). Alternately, a number of studies have failed to demonstrate hyperplasia in animal muscle following resistance training (Gollnick et al.,1983; Barnett et al.,1980). The discrepency between these studies

may relate to differences in: 1) animal species used, 2) animal age, 3) muscle(s) studied, 4) methods used to induce hypertrophy, and 5) techniques used to assess fiber number (Gollnick et al., 1983). Indirect evidence suggests that hyperplasia in humans is not a mechanism that contributes to skeletal muscle hypertrophy following heavy resistance training (MacDougall et al.,1984; Saltin & Gollnick,1983). The large variability in muscle fiber number between and within trained and sedentary subjects has been attributed to genetic variation (MacDougall,1986) although some authors suggest that hyperplasia may also occur (Tesch and Larsson,1982; Tesch, 1987). For a more detailed discussion of this topic the reader can consult two reviews (MacDougall,1991; Taylor & Wilkinson, 1986).

While much information has been obtained about physiological adaptations following a program of strength/ resistance training, knowledge of the mechanisms that initiate and regulate muscle hypertrophy is still rudimentary. Acute and chronic changes in muscle protein turnover (synthesis and degradation) following resistance training are of particular interest but only a handful of studies of this nature have been performed in humans. Chronic increases in quadriceps muscle protein synthesis have been documented following 3 and 12 weeks of resistance training respectively (Rennie et al.,1980; Yarasheski et al.,1990). Muscle protein degradation has been assessed following resistance training by urinary

excretion of 3-methylhistidine (3-MH). Methylhistidine is an amino acid that is found in actin and the myosin heavy chain of white muscle. Its unique property is that it is not re-incorporated back into myofibrillar protein once it is released (Rennie & Millward,1983). Urinary 3-methylhistidine (3-MH) excretion does not appear to be acutely elevated following a single bout of resistance training (Hickson et al.,1986; Horswill et al.,1988; Paul et al., 1989). However, when resistance training is performed over a number of consecutive days 3-MH excretion increases and reaches a new plateau level (Pivarnick et al.,1990). Although studies of 3-MH excretion have provided some useful information regarding muscle protein degradation, the results are only semi-quantitative and limits as to their interpretation exist (Rennie & Millward, 1983). Information regarding mixed muscle protein synthetic rates in humans following a single session of resistance training is lacking. In addition, the time course for these changes is unknown. A model has been proposed where muscle protein synthesis is inhibited during an acute exercise session (defined as any form of contractile activity less than 6 hours in duration). The inhibition is subsequently reversed so that 1-2 hours following the termination of activity muscle protein synthetic rates are elevated above pre-exercise levels (Booth et al.,1982). The duration of this elevation is unknown but may depend on the nature of the contractile activity (Booth et al.,1982; Goldspink et

al.,1983; Rogers et al.,1979). Although this model is very general, it provides a useful framework for the study of acute changes in muscle protein synthesis following an isolated bout of resistance training.

The purpose of this introductory chapter is twofold. First, muscle hypertrophy will be discussed with reference to: 1) the methods commonly used to induce muscle hypertrophy in humans and in animals, 2) the changes that occur in gene expression and protein synthesis in animal muscle undergoing hypertrophy, and 3) some potential intracellular and extracellular signals that may play a role in stimulating muscle protein synthesis. Second, the applicability of stable isotope methodology for the study of muscle protein synthesis in humans will be discussed.

1.2 RESISTANCE TRAINING-INDUCED MUSCLE HYPERTROPHY IN HUMANS

A large number of studies have demonstrated that resistance training is an effective method for increasing the strength and mass of the major muscle groups of the body. With reference to muscles of the lower body, 60 days of unilateral isokinetic leg extension training produced selected increases in torque outputs and a 8.5% increase in the cross-sectional area of the quadriceps (Narici et al.,1989). Similarly, 10 weeks of unilateral isotonic strength training increased knee extensor torque outputs and led to increases of 21% and 18% respectively for type IIa and type IIb fiber areas (Houston

et al., 1983). In addition, a group of elite caliber strength athletes displayed a 2.5 fold greater mean fiber area of the gastrocnemius muscle compared to an age-matched sedentary control group (Alway et al.,1988). With reference to muscles of the upper body, 5 months of heavy resistance training produced a mean increase of 91% for elbow extensor strength and mean increases of 33% and 27% respectively for type II and type I fiber areas of the triceps brachii (MacDougall et al.,1979). In addition, 100 days of isometric training led to increases of 92% in isometric flexor strength and 23% in elbow flexor cross-sectional area (Ikai & Fukunaga, 1970).

The results from these studies indicate that: 1) the magnitude of muscle hypertrophy is variable and the growth process is slow, 2) increases in muscle size do not solely account for increases in strength, and 3) muscle hypertrophy can be achieved by employing isotonic, static, or isokinetic resistance training protocols. A fundamental question that arises from these observations is what is the stimulus for work-induced muscle growth? The answer to this question appears to be related to the production of mechanical tension by muscle (Booth et al.,1982; Goldberg et al.,1975; McDonagh & Davies,1984). A common element found in studies where muscle hypertrophy and strength gains occur is a training intensity that exceeds 66% of the one-repetition maximum (1RM) for any given resistance exercise (McDonagh & Davies,1984). Since the recruitment threshold of fast-twitch motor units is high

(Sale,1987), this suggests that the activation of fast-twitch (FT) muscle fibers may be an important determinant of muscle size when loads exceeding 66% of the 1RM are lifted. In addition, Goldberg's (1967) observations of a greater basal muscle protein synthetic rate in tonic compared to phasic muscle suggests that FT fibers may be more sensitive to the hypertrophy stimulus than slow-twitch (ST) fibers. In humans, the basal protein synthetic rate of tibialis anterior (a predominantly ST muscle) is greater than the basal protein synthetic rate of the quadriceps (a more homogeneous fiber composition) (Smith & Rennie,1990). Indirect evidence for this hypothesis comes from a number of resistance training studies where the relative hypertrophy of FT muscle fibers was found to be greater than ST muscle fibers (MacDougall et al.,1979; Tesch et al.,1986; Thorstensson et al.,1976). The possible intracellular/extracellular signals linking the development of mechanical tension to increases in muscle mass will be discussed later.

1.3 METHODS TO INDUCE MUSCLE HYPERTROPHY IN ANIMALS

Work-induced growth of animal muscle can be achieved by a variety of experimental interventions. The most common methods are tenotomy (Goldberg et al.,1975), passive stretch (Goldspink,1980; Laurent et al.,1978; Vandenburgh,1987), and weight lifting exercise (Goldspink & Howells,1974; Gonyea & Ericson,1976; Ho et al.,1980, Wong & Booth,1988). The degree

of similarity between the stimulus for animal muscle hypertrophy and human muscle hypertrophy depends on how well the technique approximates resistance training with respect to its ability to isolate and apply progressive overload to muscle(s).

1.3.1 Tenotomy

Tenotomy is a common method to induce muscle growth in the rat hindlimb and, although the procedure is invasive, it is simple in nature. Briefly, a muscle or muscle group is made non-functional by surgical ablation such that the remaining synergist(s) must compensate for the muscle loss. Tenotomy of the gastrocnemius can lead to increases of 30-50% in the wet weights of the synergistic soleus and plantaris muscles compared to sham-operated contralateral controls one week post-surgery (Goldberg et al.,1975; Mackova & Hnik,1973). This model appears to be very useful for the study of work-induced hypertrophy because of the minimal intervention required and the rapidity of growth. However, unlike resistance training, tenotomy provides a continuous and unforgiving stimulus for growth leading to changes in muscle size that are far greater than what has been observed following weeks to months of resistance training (Taylor & Wilkinson,1986). In addition, more than one stimulus may be present in this model. It has been shown that type II muscle fibers from rat plantaris can enlarge independently of weight

bearing following ablation of the soleus and gastrocnemius combined with hindlimb suspension but when type I fibers from this muscle were examined atrophy was evident (Michel et al.,1989). These results suggest that weight bearing is a requirement for type I fiber hypertrophy following tenotomy while type II fiber growth occurs by a different mechanism. This mechanism is believed to be related to muscle stretch (Mackova & Hnik,1973; Taylor & Wilkinson, 1986). Finally, tenotomy may produce biochemical changes in the affected muscle(s) that do not occur in human muscle that has been subjected to resistance training (Baldwin et al.,1977; Ianuzzo & Chen,1979).

1.3.2 Passive Stretch

Passive muscle stretch performed under in vivo or in vitro conditions is a potent stimulator of muscle hypertrophy. In vivo stretch of chicken patagialis muscle for 24 hours led to an 18% increase in wet weight (Barnett et al.,1980) and a mean increase of 25% in myofibril diameter following 7 days of continuous stretch (Ashmore & Summers,1981). Wing loading, by means of attached weights, for periods of 30 and 60-65 days led to mean muscle weight increases of 172% and 105% respectively in the anterior latissimus dorsi (Alway et al.,1989; Gollnick et al.,1983). Finally, an in vitro model employing continuous/intermittent stretch of embryonic skeletal myotubes has been used to study muscle hypertrophy

(Vandenburgh,1987; Vandenburgh et al.,1989). However, in vitro muscle growth rates are lower than in vivo growth rates (Goldspink et al., 1983) and muscle incubated under in vitro conditions must be maintained in a stretched position to prevent protein degradation from exceeding protein synthesis (Vandenburgh, 1987). Another dissimilarity between the stretch model and conventional resistance training is the continuous nature of the stretch stimulus. Some degree of muscle stretch occurs during eccentric and isometric contractions but the magnitude and duration is small compared to the stretch imposed on animal muscle.

1.3.3 Weight Training

Experimental protocols where animals perform activities which mimic resistance training offer advantages over tenotomy and stretch models. Unfortunately, only a few of these studies have been performed. Goldspink & Howells (1974) trained 4 groups of male hamsters to obtain food rewards by pulling down on a counterweighted food basket. The training lasted 5 weeks and was progressive. Significant increases in the mean fiber areas of the biceps brachii ranged from 17.7% in the 65 week old group to 35.6% in the 8 week old group. Similar increases in the mean fiber areas of the extensor digitorum longus were also found. Wong & Booth (1988) electrically stimulated the lower leg muscles of rats to contract concentrically against a weighted pulley bar.

Training was performed over a 16 week period and consisted of 4 sets of 6 repetitions per session at a frequency of 2 sessions per week. Following training, muscle wet weights of the trained plantar flexors were 13-18% heavier than the corresponding contralateral control muscles. Additional studies, where animals have been operantly conditioned to lift weights, will not be discussed because hypertrophy was achieved largely by hyperplasia and not by the growth of existing muscle fibers (Gonyea et al.,1986; Ho et al,1980). The muscle fiber hyperplasia that was documented in these studies may have been due to the animal species used, the resistance training protocol, and possible sources of error associated with fiber counting.

1.4 MOLECULAR CONTROL OF MUSCLE GROWTH

The sensitivity of muscle protein synthesis to acute and chronic changes in contractile activity and various physiological perturbations suggests that this process is the primary regulator of muscle size (Golgberg et al.,1975; Millward et al.,1976; Smith & Rennie,1990). In addition, in spite of major quantitative differences in the magnitude of the hypertrophy response between animal and human models, similar qualitative mechanisms appear to control the rates of muscle protein synthesis.

The synthesis of nascent polypeptides from precursor DNA in eukaryotic cells is a complex process that is subject

to extensive regulation. Briefly, 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). The hnRNA transcript is modified in the nucleus and is subsequently released into the cytoplasm as messenger RNA (mRNA). In the cytoplasm mRNA can undergo degradation or be translated by the ribosomal machinery into a new protein molecule. Translation can be divided into initiation, elongation, and termination steps and requires ATP, GTP, amino acids, transfer RNA (tRNA), ribosomal RNA (rRNA), and at least 9 different initiation factors (Waterlow et al.,1978; Pain,1986).

Several control points 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 cytoplasm, 5) translation of mRNA , and 6) protein modification (Babij & Booth, 1988; Nevins,1983). Changes in the rates of transcription and translation appear to be the most important regulators of muscle protein synthesis and will be discussed in relation to changes in contractile activity.

Two indices that have been used to infer changes in gene transcription and translation respectively are protein synthetic capacity (RNA capacity) and translational efficiency (RNA activity). Protein synthetic capacity is defined as the total RNA concentration expressed relative to non-collagenous

protein content. This index is thought to reflect changes in transcription because of a high correlation between muscle protein synthetic rates and total RNA content (Millward et al.,1973). Approximately 80% of total RNA is ribosomal RNA and therefore increases in protein synthetic capacity are likely to be due in part to increases in ribosome number. Translational efficiency (RNA activity) is expressed as protein synthetic rate relative to 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).

Acute and chronic changes in contractile activity have been shown to influence the transcription and translation of specific muscle proteins. For example, chronic electrical stimulation of the rabbit peroneal nerve at 10 Hz led to increases in mRNA coding for carbonic anhydrase III and cytochrome b and decreases in fast myosin heavy chain mRNA and aldolase mRNA (Brownson et al.,1988; Williams et al.,1986). These results are consitent with fast to slow transitions in muscle phenotype following chronic electrical stimulation (Pette & Vrbova,1985). When contractile activity is greatly diminished, as occurs during hindlimb immobilization, muscle begins to atrophy. Muscle atrophy is initiated by a reduction in muscle protein synthesis and appears to be regulated by an acute decrease in translational efficiency (Booth & Seider,

1979). In addition, immobilization for 7 days produces marked decreases in the contents of total muscle RNA and mRNA coding for selected muscle proteins (Babij & Booth,1988; Howard et al.,1989). This suggests that transcriptional control becomes increasingly important during chronic immobilization. The reversal of disuse-atrophy by weight bearing and combinations of weight bearing plus exercise have confirmed that acute changes in muscle protein synthesis are regulated by a translational mechanism. Such changes occur before any effects on gene transcription can be detected (Booth,1989; Morrison et al.,1987; Tucker et al.,1981). This strategy for controlling muscle protein synthesis also applies to animal muscle undergoing work-induced hypertrophy (Laurent et al.,1978; Wong & Booth,1990a;1990b).

Few studies have examined changes in protein synthesis in overloaded animal muscle. A 104% increase in muscle protein synthesis was found 1 day following the application of continuous stretch to the anterior latissimus dorsi muscle in fowl (Laurent et al.,1978), while tenotomy of the rat gastrocnemius led to a 45% increase in soleus protein synthesis one day post-surgery (Goldspink et al.,1983). In addition, protein synthesis was elevated 3 hours following the initiation of a 20% stretch-relaxation cycle designed to stretch avian pectoralis myotubes in vitro for a total of 60 seconds every 30 minutes (Vandenburgh et al.,1989). Acute increases in muscle protein synthesis, following tenotomy or

passive stretch, are mediated by increases in translational efficiency (Barnett et al.,1980; Goldspink,1977; Goldspink et al.,1983; Laurent et al.,1978). On the other hand, chronic increases in muscle protein synthesis are due primarily to increases in protein synthetic capacity and this is reflected by greater total RNA concentrations in hypertrophied versus control muscle (Barnett et al.,1980;Goldberg et al.,1975). Increases in DNA content have also been observed in overloaded muscle but the significance of this finding, in relation to muscle hypertrophy, is unclear (Goldberg et al.,1975). The source of the new DNA may be from connective tissue cells or from proliferation of satellite cells (Goldberg et al.,1975; Schiaffino et al.,1972).

The mechanisms that regulate the rates of transcription and translation of specific muscle proteins are poorly understood. Sobel & Kaufman (1970) observed an elevated RNA polymerase activity in soleus muscle undergoing hypertrophy 48 hours following tenotomy of the gastrocnemius. RNA polymerase is the enzyme that catalyzes the start and sequential addition of bases to form a mRNA transcript from a given DNA sequence. More recent information about transcription has been obtained with the discovery of a DNA base sequence that is highly conserved in genes coding for several muscle proteins. This sequence, known as a" CArG box" appears to be necessary for the transcription of cardiac α-actin, skeletal α-actin and myosin light chain 2 (Booth,

1983). The CArG box may be regarded as a promoter because of its upstream location from the start site of transcription. Promoters are required for accurate and efficient initiation of transcription (Maniatis et al.,1987). Proteins that bind to promoters, known as trans factors, can up-regulate or down-regulate transcription. Proto oncogene proteins and proteins from myogenic regulatory genes from the MyoD family have been proposed as possible trans factors (Booth,1989; Olson,1990; Schonthal,1990). The induction of these factors during and following exercise remains to be determined. The regulation of translation will be discussed in subsequent sections.

1.5 REGULATION OF MUSCLE PROTEIN SYNTHESIS BY CELLULAR SIGNALS

1.5.1 Amino Acids

An increased uptake of specific amino acids into muscle has been demonstrated in response to tenotomy, passive stretch and electrical stimulation. Goldberg and Goodman (1969) observed an increased uptake of the amino acid analogue aminoisobutyric acid (AIB) into overloaded plantaris and soleus muscle 4 days following tenotomy of the gastrocnemius. Similarly, [^{3}H] AIB uptake was increased by chronic stretch of embryonic avian myotubes and the administration of ouabain (a sodium channel blocker) inhibited this response (Vandenburgh & Kaufman,1982). Myotube stretch was associated with a 60-70% increase in the Vmax of the sodium pump with no apparent

change in the Km (Vandenburgh & Kaufman ,1981). These results suggest that sodium pump activity is stimulated by tenotomy/passive stretch and is required for the uphill transport of specific amino acids. To date, amino acid transport systems have not been characterized in human muscle but have been described for rat muscle (Hundal et al.,1989). Evidence suggests that only a few amino acids enter muscle by a sodium dependent mechanism (Smith & Rennie,1990). In addition, it is not known to what extent an acute bout of resistance training affects amino acid transport across working muscle. Isometric muscle contractions produced in the rat hindlimb by electrical stimulation have been shown to increase the uptake of the branched chain amino acids (Bylund-Fellenius et al.,1984).

Increased amino acid transport into muscle has been postulated to regulate protein turnover (Goldberg et al.,1975; May & Buse,1989). However, a number of observations do not support this hypothesis. First, the rate of entry of most of the 20 amino acids is likely to be greater than the rate of their intracellular metabolism (Smith & Rennie,1990). Second, the Km values for aminoacyl tRNA synthetases have been found to be lower than the intracellular concentrations of their respective amino acids (Tyobeka & Manchester,1985). This suggests that aminoacyl tRNA synthetases are saturated at physiological concentrations of intracellular amino acids. Third, an increased influx of the branched chain amino acids

into contracting muscle was accompanied by a depression in muscle protein synthesis (Bylund-Fellenius et al.,1984). This study also demonstrated that intracellular concentrations of 12 additional amino acids did not change appreciably during the contraction protocol compared to resting conditions. This was interpreted as indicating that amino acid levels within muscle did not influence protein synthesis. In spite of these observations, certain amino acids may modulate protein turnover in vitro and in vivo.

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. The administration of leucine to rats at physiological concentrations has been shown to stimulate protein synthesis and inhibit protein degradation in incubated diaphragm muscle (Fulks et al.,1975; Tischler et al.,1982). The mechanism for leucine's anabolic actions in muscle does not appear to be related to an enhanced leucyl-tRNA charging or a metabolite from leucine degradation (Tischler et al.,1982). Evidence suggesting that leucine increases ribosomal aggregation and affects peptide chain initiation has been obtained in heart muscle (Rannels et al.,1977). The modulation of whole body protein turnover by leucine infusion in humans is not always apparent or consistent with leucine's anabolic actions in

animal muscle (May & Buse,1989). In addition, methodological limitations have made it impossible to determine the precise role of this amino acid in the control of whole body and muscle protein turnover.

Recent work has established glutamine as a potential regulator of muscle protein turnover. Glutamine is a non-essential amino acid that accounts for the greatest percentage of the total intracellular free amino acid pool (Rennie & Scislowski,1989). A positive correlation between intracellular glutamine concentration and the fractional muscle protein synthetic rate has been demonstrated in humans during states of protein deficiency, starvation, and corticosteroid administration (Rennie & Scislowski,1989). More convincing evidence has been obtained from the manipulation of intracellular glutamine concentrations in the rat hindlimb preparation. An increase in muscle glutamine concentration was accompanied by increases in muscle protein synthesis. This occurred with or without insulin, although a greater effect was achieved with its addition (Rennie & Scislowski,1989). In addition, glutamine efflux from 4 day post- denervated rat hindlimb muscle increased and was accompanied by a significant reduction in the weight of the soleus and gastrocnemius (Hundal et al.,1990). 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 & Manchester,1985). A

possible role for glutamine in stimulating increases in muscle protein following resistance training remains to be determined.

1.5.2 Changes in Cellular Energy Levels

In its simplest analogy, skeletal muscle can be considered as a machine that converts the chemical energy stored in adenosine triphosphate (ATP) into mechanical work and heat. The rate of ATP hydrolysis is dependent on the metabolic characteristics of the muscle fiber types. Fast-twitch (FT) muscle fibers possess a high myosin ATPase activity and a high glycogenolytic capacity to generate ATP whereas slow-twitch (ST) muscle fibers possess low ATPase activity and a low glycogenolytic capacity to generate ATP (Saltin & Gollnick,1983). These differences account for a high shortening velocity and a high rate of ATP turnover in FT compared to ST fibers. It has been estimated that 70% of the ATP used for excitation-contraction coupling during concentric muscle contractions is required by the contractile apparatus while the remaining 30% is used mainly by the calcium pump (Rall,1988). Protein synthesis is also an energy requiring process. At least 5 molecules of ATP or its equivalent are needed for the incorporation of a single amino acid into a nascent polypeptide (Newsholme & Leech,1983). If protein synthesis and excitation-contraction coupling derive their energy sources from a common pool, then decreases in this

energy pool through its utilization for contractile activity may impair the ability to synthesize protein. Alternately, a substrate/product from a metabolic reaction could conceivably affect muscle protein synthesis.

Several studies have examined the relationship between muscle protein synthesis and changes in cellular energy levels during periods of increased contractile activity or following experimental manipulations designed to deplete energy reserves. Pain & Manchester (1970) observed a decrease in protein synthesis in the extensor digitorum longus following 90 minutes of in vitro electrical stimulation. During the final 30 minutes of the stimulation protocol, ATP levels declined by 42% compared to unstimulated control muscles. Similarly, Bylund-Fellenius et al.(1984) found large decreases in the protein synthetic rates of the tibialis anterior, gastrocnemius, and plantaris muscles following 10 minutes of isometric contractions induced by electrical stimulation. These changes were accompanied by decreases in muscle ATP and PCr content and the ATP/ADP ratio. Significant positive correlations were obtained between protein synthetic rates of the white portion of the gastrocnemius/tibialis anterior and muscle contents of ATP, PCr, and the ATP/ADP ratio. The soleus, a predominantly slow-twitch muscle, did not exhibit changes in protein synthesis or in energy metabolite levels. After 10 minutes of recovery, protein synthetic rates returned towards baseline levels as did ATP, PCr, and the ATP/ADP

ratio. Acute ischemia of the rat hindlimb, induced by tourniquet application for 45 minutes, resulted in significant reductions in gastrocnemius protein synthesis for up to 1 hour following reperfusion (MacLennan & Rennie,1989). In addition, the gastrocnemius PCr content was depressed by 56% and did not return to baseline levels until 40 minutes after tourniquet removal. The mechanism responsible for these changes might relate to alterations in polyribosomal profiles since ribosomal aggregation was depressed 10 minutes into the post-ischemic period and did not recover until 30 minutes later.

Phosphocreatine splitting and changes in the ATP/ADP ratio of the cell have been proposed as regulators of muscle protein synthesis. The free ATP content in human skeletal muscle is low (4-5 mmol/kg wet weight) (Saltin & Gollnick, 1983) and can only provide enough energy for a few maximal contractions. A second source of readily available ATP comes from the phosphorylation of ADP by phosphocreatine (PCr). This reaction is catalyzed by creatine phosphokinase and also results in the formation of creatine (Cr) (Newsholme & Leech, 1983). The discovery of localized specific isozymes of CPK on mitochondria, myofibrils, and ribosomes suggests that energy translocation via the creatinephosphokinase reaction may not be used solely for the purpose of regenerating ATP for muscle contraction (Bessman & Savabi,1990). The proposal of a phosphocreatine energy shuttle, where the production of PCr

in the mitochondria (via mitochondrial CPK) is used to phosphorylate ADP to ATP at other cell components such as ribosomes, has received support. The addition of creatine to embryonic muscle has been shown to selectively increase myofibrillar protein synthesis (Ingwall,1976; Rogozkin,1976). In addition, the use of a specific CPK inhibitor, 2,4-dinitrofluorobenzene, resulted in a 70% reduction in protein synthesis in the rat diaphragm (Carpenter et al.,1983). The rate of hydrolysis of PCr in type I versus type II muscle fibers may be an important determinant of Cr availability during and following repetitive high intensity muscle contractions. Fast-twich fibers possess a greater absolute amount of PCr and have higher CPK activity than slow-twitch fibers (Newsholme & Leech ,1983; Tesch et al.,1989). The relative decline in PCr content of the vastus lateralis was found to be 69% in FT and 59% in ST fibers following the completion of 30 maximal isokinetic leg extensions. In addition, PCr recovery was higher in ST compared to FT fibers after 60 seconds of rest (Tesch et al.,1989).

Changes in the ATP/ADP ratio induced by contractile activity or by in vitro manipulation of energy stores has been shown to affect protein synthesis. Compared to control, a 25% decrease in the ATP/ADP ratio was found in electrically stimulated extensor digitorum longus muscle. This was accompanied by a decrease in protein synthesis (Pain & Manchester,1970). In addition, Bylund-Fellenius et al. (1984)

found a positive correlation between protein synthesis in the tibialis anterior/ white portion of the gastrocnemius and the ATP/ADP ratio during rest and following 10 minutes of isometric muscle contractions. The GTP/GDP ratio, which is analoguous to the ATP/ADP ratio, is a regulator of the ternary protein synthesis initiation complex ([Met-tRNA$_f$·GTP·eIF-2]) in a reticulocyte cell model (Walton & Gill,1976). GDP was found to be a competitive inhibitor of GTP binding to the ternary protein synthesis initiation complex. This complex is formed when GTP binds to initiation factor eIF-2 followed by its association with Met-tRNA$_f$ (Pain,1986). Eukaryotic initiation factor 2's affinity for GDP is approximately 100 fold greater than for GTP. This suggests that small changes in the GTP/GDP ratio can result in significant changes in mRNA translation. GTP is formed by the phosphorylation of GDP with ATP in a near-equilibrium reaction catalyzed by nucleoside diphosphate kinase (Newsholme & Leech,1983). Therefore, a sensitive mechanism exists to control the rate of protein synthesis when the ATP/ADP ratio changes. Heavy exercise is known to reduce the muscle cell ATP/ADP ratio and to increase the cellular free Pi levels (Newsholme & Leech,1983). Phosphorylation is an important intracellular signalling mechanism (Rana & Rokin,1990; Sibley et al.,1987) and has been shown to play a role in the regulation of protein synthesis. Initiation factor eIF-2 is a substrate for protein kinases and phosphatases. When eIF-2 is phosphorylated protein synthesis

is decreased due to ribosomal disaggregation and a fall of 43s initiation complexes (Pain,1986). Thus, phosphorylation of eIF-2 by changes in the free cellular Pi levels in muscle may play an important role in the acute regulation of muscle protein synthesis.

1.5.3 Prostaglandins

Prostaglandin release from skeletal muscle has been proposed as a modulator of muscle protein turnover. An increased incorporation of [^{3}H] inositol into phosphatidyl inositol was demonstrated in overloaded soleus and plantaris muscle 2 hours following tenotomy of the gastrocnemius (Jablecki et al., 1977). This observation established a possible link between membrane phospholipid turnover and muscle hypertrophy. The prostaglandins are a group of small molecules that are synthesized from membrane phospholipids in virtually all mammalian cells. They are potent regulators of several physiological responses and are classified as hormones because they bind to specific intracellular/extracellular receptors and can function in a paracrine and/or autocrine manner (Norman & Litwack,1987). The immediate precursor for prostaglandin synthesis, arachidonic acid, is derived from membrane phospholipid hydrolysis by mechanisms thought to involve diacylglycerol, phospholipase C, and phospholipase A_2 (Rana & Hokin,1990; Billah & Anthes,1990). Two members of the prostaglandin family, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2), have been linked to the rates of

muscle protein synthesis and degradation respectively (Reeds et al.,1987). Studies have established a close temporal association between increases in muscle protein synthesis and increases in $PGF_{2\alpha}$ release from muscle. Palmer et al. (1983) demonstrated that 90 minutes of intermittent stretch of rabbit forelimb muscle produced increases of 70% and 105% respectively for protein synthesis and $PGF_{2\alpha}$ release. In addition, a significant positive correlation between $PGF_{2\alpha}$ release and protein synthesis was found. Similarly, Vandenburgh et al. (1990) found that intermittent stretch of avian myoblasts resulted in an initial period of increased protein degradation and PGE_2 release. However, by 48 hours into the stretch protocol, increases in protein synthesis and $PGF_{2\alpha}$ efflux were accompanied by a return of PGE_2 efflux to that of unstretched control myoblasts.

The mechanisms linking muscle tension to the release of membrane-bound phospholipids and their conversion to arachidonic acid and $PGF_{2\alpha}$ and PGE_2 are poorly understood. In addition, the exact nature of how $PGF_{2\alpha}$ and PGE_2 stimulate muscle protein synthesis and degradation respectively is unknown.

Diacylglycerol, the precursor of arachidonic acid, is one molecule of a bifurcating pathway that is thought to play an extremely important role in cellular signalling (Berridge, 1987; Rana & Hokin,1990). The other molecule, inositol triphosphate (1,4,5-IP_3), is a mobilizer of intracellular

calcium (Rana & Hokin,1990). The binding of growth factors to cell surface receptors has been shown to stimulate the production of diacylglycerol and to activate other messenger molecules such as protein kinase C. Protein kinase C and molecules from diacylglycerol metabolism have been linked to increased transcription of the protooncogenes c-myc and c-fos (Rana & Hokin,1990; Schontal,1990). $PGF_{2\alpha}$ acts as a mitogen when it is added to fibroblast cells in culture (Rana & Hokin,1990) and therefore may have a role in controlling cell proliferation. A role for $PGF_{2\alpha}$ in activating muscle satellite cell proliferation, in response to muscle damage, remains to be determined.

1.5.4 Muscle Damage, Growth Factors, and Satellite Cell Activation

It is well known that a bout of unaccustomed physical activity can lead to muscle fiber damage and delayed-onset muscle soreness lasting for several days (Ebbeling & Clarkson,1989; Newham,1988). Muscle fibers are believed to be damaged by mechanical disruption due to frequent and/or high intensity contractions (expressed as a % of the 1 RM). In addition, muscle damage can occur following isometric, concentric, or eccentric muscle contractions (Clarkson et al.,1986) although it is acknowledged that eccentric exercise produces the greatest damage (Stauber,1989). Indices of muscle damage include morphological abnormalities in sarcomere

structure from muscle biopsy samples (Friden,1984), a decrease in maximal voluntary force output (Clarkson & Tremblay,1988; Newham et al.,1987), elevations in the circulating levels of soluble muscle enzymes (creatine kinase in particular) (Ebbeling & Clarkson,1989), and inflammation and delayed-onset muscle soreness (Newham,1988). Resistance exercise usually involves the performance of a large number of high intensity muscle contractions per training session and this could result in muscle damage. If this is the case, does muscle damage stimulate the hypertrophy process or does it merely stimulate the synthesis of enough contractile protein to repair the damaged fibers? There is evidence that muscle damage can induce the release of specific mitogens which stimulate satellite cell proliferation. This forms the basis for a possible role of contractile protein damage in stimulating muscle growth.

Myofiber damage, resulting from eccentric muscle contractions, is characterized by focal disruptions in the banding pattern of muscle fibers (Friden,1984). Friden (1983) demonstrated that these disruptions were found primarily in type II fibers following a bout of eccentric bicycle exercise. In addition, he observed that muscle damage was associated with decreases in maximal voluntary isokinetic leg extensor torque output. The force loss was greatest at the highest test velocities and was apparent as early as 20 minutes post-exercise and persisted for up to 6 days. An interesting

finding was that polyribosome clusters were found close to the damaged fibers 3 days post-exercise. This suggested that muscle repair was proceeding. In addition, Newham et al. (1987) found large decreases in maximal voluntary isometric elbow flexor force following bouts of maximal eccentric elbow flexor contractions. The authors postulated that force loss was due to mechanical disruption and subsequent degradation of a labile pool of muscle fibers.

Another indicator of muscle damage is an increase in serum/plasma creatine kinase (CK) levels. Creatine kinase is a cytoplasmic enzyme that catalyzes the phosphorylation of ADP to ATP. The size (80,000 Da) of this enzyme does not allow it to pass through the plasma membrane under normal circumstances (Hortobagyi & Denaham,1989). Creatine kinase entry into the circulation is thought to be indicative of muscle membrane damage but it does not provide a quantitative assessment of the amount of damage. Increases in plasma/serum CK levels have been documented following concentric, isometric, or eccentric muscle activity (Clarkson et al.,1986). In addition, the post-exercise time course and magnitude of CK increase can vary tremendously between subjects (Ebbeling & Clarkson,1989). It has been shown that a single bout of eccentric exercise can exert a protective effect on muscle fiber integrity following a similar bout of exercise performed several weeks later (Clarkson & Tremblay,1988; Newham et al.,1987). This conclusion is based on a smaller plasma CK increase, less

post-exercise muscle pain and tenderness, and a smaller relative decline in maximal voluntary isometric force following the second exercise session compared to the first (Clarkson & Tremblay,1988). It is not known how muscle adapts to resist further damage but it is possible that myofiber repair is accompanied by the synthesis of extra contractile material. Because a program of progressive resistance training emphasizes muscle overload, it is reasonable to assume that myofiber damage occurs. Paul et al. (1989) found that serum CK levels were elevated in a group of experienced weight trainers 12 and 24 hours following a single bout of resistance training.

A link between muscle fiber damage, growth factor release, and satellite cell activation has been established. Growth factors are simple peptides with molecular weights in the 3,000-25,000 Da range that have broad biological effects in many cell types (Florini & Magri,1989). These agents have been classified as hormones because they bind to specific cell surface receptors and can initiate a cascade of intracellular signalling events analogous to the peptide hormones. Growth factors have greater potency (at concentrations in the ng/ml range) than endocrine hormones and can be synthesized in many cell types. In addition, growth factors can stimulate the cells from which they originate or neighboring cells (autocrine/paracrine function) (Norman & Litwack,1987). Growth factors are classified as mitogens because they stimulate 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 are a population of dormant muscle cells that are located just beneath the surface of a muscle fiber (White & Esser,1989). Satellite cells constitute 2-10% of the total fiber-associated myonuclei in young and adult vertebrates and act as a source of reserve stem cells. Satellite cells can be activated and induced to migrate, proliferate, and differentiate by a variety of stimuli including exercise, muscle damage, and mitogens (Bischoff,1986; Schultz,1989; White & Esser,1989; Yamada et al.,1989).

Recent studies have demonstrated that work-induced hypertrophy can increase growth factor release. Vandenburgh (1983) and Summers et al. (1985) showed that growth factors, isolated from chronically stretched muscle, were required to maintain increased growth rates and could stimulate satellite cell proliferation in other cell lines. In addition, the mRNA contents of insulin-like growth factors I (IGF-I) and II (IGF-II) increased in overloaded soleus and plantaris muscle following tenotomy of the gastrocnemius (DeVol et al.,1990). IGF-I has been shown to be a potent stimulator of satellite cell proliferation and to increase the mRNA content of the protooncogene c-fos in a muscle cell line (Ong et al.,1987; Sara & Hall,1990). Yamada et al. (1989) found that work-induced hypertrophy of the soleus and plantaris was

accompanied by increases in fibroblast growth factor (FGF) activity and content. In addition, the authors found that FGF was stored in the extracellular matrix of muscle. Extracellular matrix disruption, following a bout of eccentric exercise, has been documented in humans (Stauber et al.,1990). Whether this could have resulted in the release of FGF and subsequent satellite cell activation is a matter of speculation. Finally, the role of satellite cells in human muscle hypertrophy is unknown. It is unlikely that satellite cells account for all muscle growth since the satellite cell population is small and declines with age (Schultz,1989). Further study of muscle damage, and growth factor/satellite cell involvement in work-induced hypertrophy is warranted.

1.5.5 Hormonal Influences

A number of studies have demonstrated that acute increases in the concentrations of anabolic hormones such as testosterone and growth hormone can occur following an isolated bout of heavy resistance training (Kraemer et al.,1990; Vanhelder et al.,1984; Weiss et al.,1983). It is unlikely, however, that acute increases in anabolic hormone levels is the primary stimulus responsible for increases in muscle protein synthesis and muscle hypertrophy. This conclusion is based on the facts that: 1) work-induced hypertrophy can occur in hypophysectomized and castrated animals suggesting that more important signals, besides

hormones, stimulate muscle growth (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 (exercised and unexercised), and 3) supraphysiological doses of hormones such as insulin or growth hormone do not appear to enhance muscle protein synthesis in healthy humans (Smith & Rennie,1990; Yarasheski et al.,1990) although evidence for a growth-promoting effect of anabolic steroids has been obtained (Boone et al.,1990; Griggs et al.,1989). It is possible that muscle subjected to resistance training may become more sensitized to increases in anabolic hormone levels but how this might occur is unknown. Finally, it has been suggested that chronic elevations in testosterone and the testosterone/cortisol ratio may provide a more optimal anabolic environment for muscle growth (Hakkinen et al.,1987; Hakkinen et al.,1988). More research is required to define the role of hormones in inducing muscle hypertrophy.

1.6 STABLE ISOTOPE METHODOLOGY

The magnitude of skeletal muscle growth, following a program of heavy resistance training, has been routinely assessed by the needle biopsy technique and, more recently, by computerized tomographic scanning (CT scan) (Cureton et al.,1988; Narici et al.,1989). Muscle tissue obtained by

needle biopsy can be examined in cross-section for changes in fiber size and for the characterization of the different fiber types based on ATPase staining (Saltin & Gollnick,1983). In addition, CT scans of limb segments provide a non-invasive measure of muscle group cross-sectional areas when corrected for fat and bone tissue content. A combination of these techniques, over a prolonged period of time, provides a picture of net muscle growth or atrophy at the fiber and whole muscle level. However, these techniques do not indicate how muscle protein synthesis and degradation rates are affected following acute or chronic resistance training. A large body of literature has accumulated suggesting that muscle protein synthesis is more sensitive to physiological stimuli than protein degradation (Millward et al.,1976; Smith & Rennie, 1990). This provides a rationale for the study of muscle protein synthesis in resistance-trained subjects.

A resurgence in the use of stable isotopes to examine the fate(s) of several biological molecules has occurred during the past quarter century. This has been due to technological advances in gas chromatography/mass spectrometry, the widespread availability of ^{18}O, ^{2}H, ^{13}C, and ^{15}N labelled tracers, and an increased awareness of the potential health hazards associated with radioactive isotopes (Thompson et al.,1989; Wolfe,1984). As a result, a large number of recent studies have examined whole body protein turnover and muscle protein synthesis in humans during a

variety of physiological states. Whole body protein synthesis is depressed during conditions of fasting and disease (Bennet et al.,1989; Rennie et al.,1982; Rennie,1985; Rennie,1986) and is increased by feeding and following aerobic exercise (Devlin et al.,1990; Rennie et al.,1980; Rennie et al.,1982). However, in spite of a 40-50% contribution by muscle to total body weight, muscle protein synthesis (MPS) accounts for only 25-30% of whole body protein synthesis (WBPS) (Nair et al.,1988). This is due to the slow turnover rates of myofibrillar proteins compared to proteins which turn over more rapidly in tissues such as liver, kidney, spleen, and gut (Waterlow et al.,1978). Muscle is an extremely important tissue not only from a functional perspective but also because it contains the majority of the body's protein reserve (Smith & Rennie,1990). Skeletal muscle is a very "plastic" tissue and as such can atrophy or hypertrophy when a given stimulus is provided or removed. It is thus desirable to measure MPS directly since WBPS does not give quantitative information about the anabolic state of a muscle or muscle group. The effect of resistance exercise on MPS has received very little attention in spite of the value of this form of training in increasing muscle mass. The purpose of this section will be to outline the applicability of stable isotope methodology for the study of MPS in humans following an isolated bout of resistance exercise.

Before a discussion of stable isotope methodology is presented, it is useful to discuss some terminology that is frequently encountered in the literature. Stable isotopes of a given element may be 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 1.11% and 0.37% respectively (Wolfe,1984). The infusion of a labelled amino acid into the venous circulation will increase the plasma and body pool enrichments of the label above natural background levels. The increase in enrichment is usually expressed in atoms percent excess (APE) and values of 3-5 APE are typicaly achieved after 2 hours of a primed-constant infusion of 99% enriched L-[1-^{13}C] leucine in the plasma pool (Smith & Rennie,1990). This means that 3-5 out of every 100 amino acid molecules of interest are enriched with the label above background natural abundance. In addition, 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 frequently employed kinetic factors (expressed as mass of substrate per unit time). The use of stable isotopes to measure whole body protein turnover and muscle protein synthesis is contingent upon several assumptions. These are: 1) the labelled tracer is indistinguishable biologically from the naturally occurring

tracee, 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 (Thompson et al.,1989; Wolfe,1984). Evidence to support these assumptions has been presented and can be obtained in two monographs (Waterlow et al.,1978; Wolfe,1984).

The most common method of administering a ^{13}C labelled amino acid is by the primed-constant infusion technique. This involves the injection of a bolus dose of the ^{13}C amino acid (usually 1 mg/kg of a 99% ^{13}C labelled amino acid dissolved in normal saline) followed by a constant infusion of the label for several hours (1 mg/kg/hour). The purpose of the bolus dose is to rapidly elevate the enrichment of the plasma and tissue pools so that the time to reach an isotopic steady-state is shortened. An isotopic steady-state exists when the rate of appearance of the label in plasma is equal to its rate of disappearance into tissue, i.e. when Ra=Rd (Wolfe,1984). Accurate measures of whole body protein turnover and muscle protein synthesis can only be made when Ra=Rd because if Ra<Rd protein synthesis will be overestimated while if Ra>Rd protein synthesis will be underestimated. It has been shown that prolonged infusion of a given labelled amino acid tracer 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 MPS. Fortunately, the infusion time for a primed-constant infusion of a labelled amino acid such as leucine is short (6-10 hours) compared to the turnover rates of myofibrillar proteins (Waterlow et al.,1978). The choice of an amino acid, its label and label position and the infusion doses and times depend on: 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 (Smith & Rennie,1990; Thompson et al.,1989).

Tyrosine and phenylalanine are not metabolized in skeletal muscle and, as a result, their only fates are incorporation into and release from muscle protein. These amino acids have been employed extensively to study muscle protein turnover in animals (Goldberg et al.,1975) but have been used infrequently in the study of human protein metabolism. The disappearance of radiolabelled phenylalanine from the arterial side of a muscle group in man has been used as an index of protein synthesis while its appearance in venous blood has been used as an index of protein degradation (Barrett & Gelfand,1989). However, arterio-venous difference studies are limited because the calculation of muscle protein synthesis and degradation requires accurate measures of blood

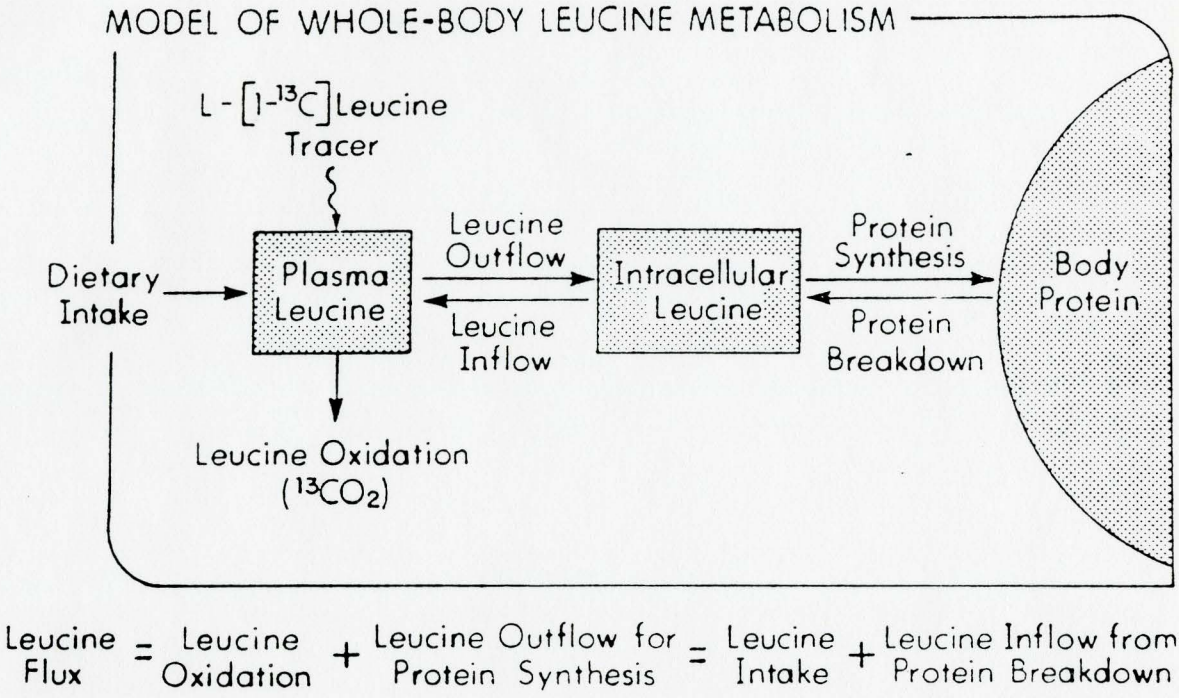
flow and the specific activity of arterial phenylalanine is assumed to represent the specific activity of phenylalanyl-tRNA (the precursor for protein synthesis) (Smith & Rennie,1990).

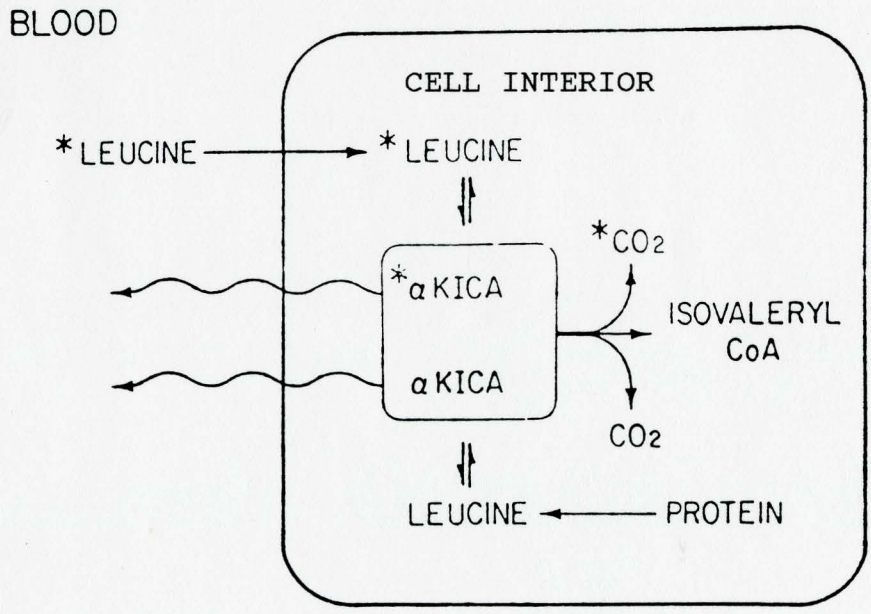
L-[1-^{13}C] labelled leucine has been the amino acid of choice for studies examining whole body protein turnover and muscle protein synthesis. Leucine is an essential amino acid and therefore cannot be synthesized de novo in body tissues (Newsholme & Leech,1983). This implies that the only sources of this amino acid are dietary and from protein degradation. Leucine is also one of the three branched chain amino acids that is oxidized primarily in muscle (Odessey et al.,1972). Leucine is an appropriate choice to study MPS because its free intracellular concentration in muscle accounts for only 0.41% of the total free amino acid pool while its concentration in muscle protein accounts for 8-10% of the total bound amino acid pool (Smith & Rennie,1990). The importance between the difference in the free intracellular leucine concentration and its protein bound concentration in muscle is apparent when consideration is given to 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. In addition, the large size of the leucine pool in muscle functions as a metabolic sink into which label is incorporated but released at a slow rate due to the slow turnover of muscle protein .

The reciprocal pool model of leucine metabolism is an attempt to obtain valid and accurate measures of muscle protein synthesis in humans. Leucine is rapidly transaminated to α-ketoisocaproic acid (α-KIC) upon entry into muscle. The reaction, catalyzed by branched chain aminotransferase, is readily reversible such that α-KIC can be reaminated back to leucine (Newsholme & Leech,1983). Alpha-KIC can also be irreversibly decarboxylated at the α-carbon to yield isovaleryl CoA. Thus, the infusion of L-1-^{13}C leucine permits the recovery of the label in expired CO_2 to measure leucine oxidation and in muscle protein obtained from biopsy samples. The increase in incorporation of ^{13}C leucine in muscle samples obtained several hours apart allows the calculation of an absolute value for muscle protein synthesis. However, it is desirable to express MPS relative to the true precursor of synthesis, aminoacyl-tRNA, because the labelling of intracellular leucine pools may not be uniform due to compartmentation (Schneible et al.,1981; Smith & Rennie,1990). Fractional muscle protein synthetic rates, expressed as % of protein synthesized/hour (%/h), have been calculated in several studies. The equation describing MPS (%/h) is the absolute change in leucine incorporation (in APE) divided by the product of plasma α-KIC enrichment (APE) and the incorporation time between 2 biopsies (hours). This is multiplied by a factor of 100 to obtain a value expressed in

%/h. Typical values for mixed muscle protein synthesic rates, in healthy postabsorptive humans, range from 0.04-0.06 %/h (Smith & Rennie,1990). Whole body distribution and intracellular metabolism of leucine is described in figure 1. The isolation of leucyl-tRNA or other aminoacylated-tRNA's is a difficult task due to the large tissue requirements (grams) and the rapid turnover rates of these complexes (2-10 seconds) (Rannels et al.,1977; Smith & Rennie, 1990; Waterlow et al.,1978). Because of these methodological limitations, plasma α-KIC labelling has been employed to approximate leucyl-tRNA labelling. The reasons for this choice are: 1) α-KIC readily traverses the muscle cell membrane such that an equilibrium is established between intracellular and extracellular α-KIC, 2) α-KIC can only be formed from leucine intracellularly and therefore the dilution of its ^{13}C label should reflect intracellular metabolism of leucine, and 3) plasma α-KIC can be isolated and measured by gas chromatography/ mass spectrometry (Ford et al.,1985; Rocchiccioli et al.,1981). Recently, leucyl-tRNA has been measured in human muscle samples taken from postabsorptive surgical patients (Smith & Rennie,1990). A comparison between leucyl-tRNA enrichment, plasma α-KIC enrichment, and intracellular leucine enrichment showed that plasma α-KIC and intracellular leucine enrichments were 105% and 83% of leucyl-tRNA enrichment respectively. These results suggest that plasma α-KIC labelling closely approximates leucyl-tRNA labelling.

FIGURE 1: WHOLE BODY AND INTRACELLULAR LEUCINE METABOLISM





Few studies have examined protein synthetic rates in human skeletal muscle in response to muscle disuse or resistance training. Following 5 weeks of unilateral leg immobilization, Gibson et al. (1987) found a 26% decrease in protein synthesis in the vastus lateralis of the affected leg compared to the contralateral non-immobilized leg. In addition, Gibson et al. (1988) found that 1 hour of electrical stimulation per day, at an intensity corresponding to only 5% of maximal voluntary contraction, could prevent decreases in protein synthetic rates in the vastus lateralis. With reference to resistance training, Tarnopolsky et al. (1991) concluded that whole body protein synthesis was not affected by an isolated bout of circuit resistance training for up to 2 hours post-exercise. This suggests that muscle protein synthesis may be unchanged during this time as well. However, caution should be exercised with this interpretation because MPS accounts for only 25-30% of whole body protein synthesis (Nair et al.,1988). Chronic increases in vastus lateralis protein synthetic rates were found following 3 and 12 weeks of resistance training respectively (Rennie et al.,1980; Yarasheski et al.,1990). However, studies examining changes in muscle protein synthesis following an acute bout of resistance training have not been performed.

1.7 SUMMARY

From the information presented, it is clear that the mechanisms regulating skeletal muscle hypertrophy, as a result of resistance training or protocols that mimic resistance training, are complex and poorly understood. It appears that a threshold level of tension production is required for growth to occur in human muscle. The link between mechanical tension and muscle growth is likely to be mediated by several interacting intracellular signals. These signals should not be viewed as discrete events but rather as part of a continuum leading to chronic increases in muscle size. Acute and chronic increases in muscle protein synthesis have been shown to be the most important causes of muscle hypertrophy. In addition, evidence has been presented to show that acute increases in muscle protein synthesis are the result of an enhanced rate of translation. Finally, muscle damage has been suggested as being a possible stimulus for muscle hypertrophy.

The purpose of this study was twofold: first, to assess the rate of biceps muscle protein synthesis in experienced resistance-trained subjects following an isolated bout of unilateral biceps resistance training. In order to describe the time course for muscle protein synthesis, one group of subjects was studied 4 hours post-exercise while another group was studied 24 hours post-exercise. The second purpose of this study was to obtain information about the mechanisms which regulate muscle protein synthesis following

an isolated bout of resistance training.

CHAPTER II

CHANGES IN MUSCLE PROTEIN SYNTHESIS FOLLOWING RESISTANCE EXERCISE

2.1 INTRODUCTION

It is well known that a program of heavy resistance training can lead to substantial increases in muscle size and strength over a course of several months (MacDougall,1986; Tesch,1987). The observed muscle hypertrophy is the result of increases in muscle fiber cross-sectional area with fast-twitch (FT) fibers attaining greater relative hypertrophy than slow-twitch (ST) fibers (MacDougall et al.,1977; Tesch,1987). Although specific adaptations have been documented in muscle morphology and biochemistry, and in the neuromuscular and endocrine systems following a program of resistance training (Kraemer,1988; Sale,1988; Tesch,1987), little is known about the mechanisms that regulate muscle growth. The production of mechanical tension by muscle is thought to be the primary stimulus for growth (McDonagh & Davies,1984). The signals, however, that link the development of tension to effect increases in muscle protein synthesis are unknown. There is a paucity of information regarding resistance training-induced changes in protein synthesis in human muscle. Although chronic

training and following 12 weeks of a whole body training program (Rennie et al.,1980; Yarasheski et al.,1990), information is lacking regarding acute changes in muscle protein synthesis and its time course in humans.

Work-induced muscle growth in animals is the result of increases in gene transcription and translation with increases in translation occurring before increases in transcription (Booth & Watson,1985; Booth 1989; Laurent et al.,1978). The cellular signals that alter gene transcription and translation are unknown but several possibilities have been proposed (Booth & Watson,1985; Booth,1988; Booth,1989).

A common belief of bodybuilders is that muscle is "torn down" so that growth can be stimulated. Heavy muscular exercise, involving an eccentric component, is a well known cause of myofiber damage (Stauber,1989) and is manifested by acute decreases in maximal voluntary force output (Friden et al.,1983), and an increased leakage of intracellular enzymes into the circulation (Ebbeling & Clarkson,1989). Because protein turnover increases with chronic resistance training, it is conceivable that muscle damage may somehow stimulate an increase in muscle protein synthesis so that damaged fibers are repaired and made more resistant to further insults. Several studies have demonstrated that a single bout of eccentric contractions can exert a protective effect on muscle so that damage and soreness are minimized following a subsequent bout of the same type of exercise (Clarkson &

Trenblay,1988; Friden,1984). In addition, activity-related or experimentally-induced muscle damage has been shown to stimulate satellite cell proliferation 24-72 hours following the insult (Schultz et al.,1989; White & Esser,1989).

The reciprocal pool model of leucine metabolism has been employed to study whole body protein turnover and muscle protein synthesis during a variety of physiological conditions (Bennet et al.,1989; Gibson et al.,1987; Halliday et al.,1988; Nair et al.,1988; Rennie et al.,1982). The primed-constant infusion of L-[1-^{13}C] leucine allows the measurement of whole body leucine flux, leucine oxidation, and muscle protein synthesis from biopsy specimens (Rennie et al.,1982; Nair et al.,1988). With regard to muscle protein synthesis, the use of plasma α-KIC enrichment to approximate the true precursor enrichment for protein synthesis, leucyl-tRNA, has received experimental support (Smith & Rennie,1990). In addition, leucine is an appropriate amino acid for the study of muscle protein synthesis because it is an essential amino acid, comprises only a small portion of the free intracellular amino acid pool, and makes up 8-10% of muscle protein (Smith & Rennie,1990).

Booth et al. (1982) have proposed a model where muscle protein synthesis is inhibited during, and 1-2 hours following, an acute bout of exercise with subsequent recovery and increases in synthetic rates occurring for an undefined period thereafter. In spite of its generality, this model

provides a useful framework for the study of human muscle protein synthesis following an single bout of heavy resistance training. Based on this model, it was hypothesized that an isolated bout of resistance training, similar to that performed by bodybuilders, would produce acute increases in muscle protein synthesis following exercise.

The purpose of this study was twofold. First, to examine changes in muscle protein synthesic rates 4 and 24 hours following a single bout of unilateral biceps resistance training in order to better understand the time course for this process. This was accomplished by the use of the reciprocal pool model of leucine metabolism combined with muscle biopsies from exercised and contralateral control arms respectively. The second purpose of this study was to examine changes in some overall markers for muscle gene transcription/translation and indices of muscle damage in order to provide possible information on how acute changes in muscle protein synthesis might occur following resistance exercise.

2.2 METHODOLOGY

2.2.1 Subjects

Twelve males, who regularly participated in weight training, served as subjects. They were advised of the risks of the study and gave written informed consent. The study was approved by the University Ethics Committee. Subjects were

assigned to either a 4 hour post-exercise group (group A) or a 24 hour post-exercise group (group B). Subjects were recruited so that the 2 groups could be equated on the basis of resistance training experience and maximal elbow flexor strength.

2.2.2 Experimental Protocol

A)Preliminary Measures

Subjects reported to the laboratory for the determination of body weight and height, maximal elbow flexor strength, and percent body fat. The testing order was the following: 1) weight and height measures, 2) one-repetition maximum strength (1 RM), 3) residual volume determination, and 4) hydrostatic weighing. Unilateral 1 RM strength of the dominant arm was determined for 3 different biceps exercises. Following a warm up protocol with a light dumbbell, 1 RM strength was determined for the biceps curl, preacher curl, and concentration curl exercises. The biceps curl was performed by having the subjects flex their elbow from resting extension to maximal flexion with the wrist kept in a supinated position. Isolation of the curling motion was ensured by having the subjects place their backs against a wall with the knees slightly bent. The preacher curl consisted of a seated curl on a low level incline bench. The angle of inclination was adjusted and recorded for each subject such that the back of the exercising arm rested comfortably on the bench. The concentration curl was performed in a seated

position with the dominant elbow placed perpendicularly against the thigh. A curling motion was then performed from full extension to just beyond 90° of flexion. Weight was added for each exercise until the subject could not successfully complete the lift. Gradations for adding weight were to the nearest kilogram. To avoid the possible effects of fatigue, subjects were given 3-4 attempts to reach their 1 RM and 1-2 minutes of rest was provided between trials and exercises. The criteria used to indicate that 1 RM strength was achieved were: 1) the ability to just complete a strict repetition of a given exercise, and 2) the inability to perform a complete repetition with a heavier weight following a successful 1 RM attempt.

Body density was determined by hydrostatic weighing with residual lung volume measured by helium dilution. Percent body fat of each subject was calculated with the Siri equation (1956) using a mean of 2 underwater density measures. Three day food records (including one weekend day) were obtained from each subject for the determination of mean energy intake using a computer program for nutrient analysis (Nutritionist 3).

B)Training Protocol

Eight subjects reported to the laboratory in the early morning hours while 4 subjects (2 from each group) reported in the late afternoon. Subjects in group A (4 hour post-exercise group) trained on the day of the leucine infusion

while subjects in group B (24 hour post-exercise group) trained the day before the leucine infusion. All subjects reported to the laboratory following 3 days of rest. The training session consisted of performing 4 sets of 6-12 repetitions of the biceps curl, preacher curl, and concentration curl respectively. All sets were performed to muscular failure and rest periods of 3 minutes were provided between sets and exercises. The amount of weight lifted and the number of full repetitions completed was recorded for each set for subsequent calculations of training intensity (% of 1 RM) and training volume (weight lifted x sets x repetitions).

C)Indices of Muscle Damage

In order to evaluate possible muscle damage, as a result of the training session, isokinetic elbow flexor strength was measured and pre/post exercise blood samples were drawn for the determination of serum creatine kinase activity. Subjects from both groups were tested for maximal elbow flexion torque of the dominant arm just prior to and 15 minutes following the completion of the resistance training session. Low and high speed strength, corresponding to velocities of 30°/s and 180°/s respectively, was recorded with an isokinetic dynamometer (Cybex II, Ronkonkoma, NY). Subjects performed 2-3 maximal concentric contractions at each velocity in a kneeling position with the arm resting comfortably on a

padded table. Subjects grasped an adjustable handle attached to the dynamometer lever arm so that the axis of the elbow joint was aligned with the axis of the dynamometer lever arm. All contractions were performed from full extension to maximal flexion. The highest peak torque from the 2-3 attempts was recorded. The velocity testing order was randomized. Blood samples were drawn from an antecubital vein from group B subjects only prior to and 22 hours following the training session. After being allowed to clot, the samples were centrifuged and the serum stored at -70°C until analysis. The 22 hour blood samples were obtained before the first set of muscle biopsies to ensure that serum CK levels reflected the biceps training session only and not damage caused by the biopsy procedure itself.

D)Feeding

Subjects received 50% of their individual mean energy intake as a defined formula diet (Ensure, Ross Laboratories, Montreal, Quebec). The feedings began 2 hours prior to the start of the leucine infusion and were given in equal aliquots every 30 minutes (17 aliquots total) during the study protocol. The rationale for keeping the subjects in a fed state during the study was to prevent a possible decrease in muscle protein synthesis because fasting has been shown to depress muscle protein synthesis (Rennie et al.,1982).

E)Leucine Infusion

The leucine infusion protocol was similar for both groups with the exception that group A was infused 0.68 ± 0.20 h following exercise while group B was infused 20.41 ± 0.24 h post-exercise. Subjects in group B were instructed to refrain from strenuous activity following the resistance training session.

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 α-KIC enrichment. A second catheter was inserted into a vein of the contralateral proximal forearm for isotope infusion. A priming dose of L-[1-^{13}C] leucine (1 mg/kg) was administered followed by a constant infusion of L-[1-^{13}C] leucine (1 mg/kg/h) for 6 hours, delivered by a calibrated Harvard syringe pump. The actual infusion times were 5.40 ± 0.71 and 6.38 ± 0.41 h (mean ± SD) respectively for groups A and B. The L-[1-^{13}C] leucine for the entire study was from the same batch (MSD Isotopes, Pointe Claire, PQ) and was confirmed to be 99% isotopic purity and sterile by the company. Batch dilutions (15 g/ml) of the isotope were made under aseptic conditions and on the day of the infusion, the isotope was further diluted with sterile saline and microfiltered immediately prior to infusion. Arterialized blood samples, for the determination of plasma α-KIC enrichment, were obtained 2 hours following the priming dose (t=0) and approximately

midway (t=+2 h) and at the end of the infusion protocol(t=+4 h). Blood was collected into heparinized tubes and centrifuged immediately. The plasma was stored at -70°C until analysis.

F)Muscle Biopsies

Percutaneous needle biopsies from the distal lateral portion of the biceps brachii were obtained under local anaesthesia 2 h following the leucine priming dose and at the end of the leucine infusion protocol (approximately 4 h following the priming dose). Two biopsies were obtained at 2 h (one from each arm) and two more were taken at the end of the infusion (one from each arm but slightly more proximal). The muscle samples were visibly dissected of fat and connective tissue and were frozen in liquid nitrogen. The samples were transferred to a -70°C freezer until analysis. The 2 hour 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)Protein, RNA, and DNA Determination

The frozen muscle samples were weighed, and lyophylized overnight. The dry muscle weights were recorded before each sample was ground to a fine powder in liquid nitrogen with a mortar and pestle. The ground muscle from each sample was transferred to tubes containing 3 ml of 0.2N ice

cold perchloric acid (PCA). The tubes were capped, vortexed, and centrifuged at 2800 rpm for 20 minutes at 4°C. The supernatant was discarded. The remaining pellet was redissolved in 5 ml of 0.2N PCA and centrifuged once more. This step was repeated. Tissue lipids were then extracted by a series of 5 ml solvent washes followed by 5 minutes of centrifugation at 2800 rpm at 4°C for each wash. The order of the washes was: 1) 1% potassium acetate in ethanol, 2) ethanol:chloroform (3:1), 3) ethanol:ether (3:1), and 4) ether. Protein was solubilized in 3 ml of 0.3M NaOH in a 37°C water bath for 60 minutes. A 50 ul aliquot of the supernatant was removed and added to 4.95 ml of 0.3N NaOH. The alkali soluble protein was transferred to clean tubes. Muscle protein content was determined by the method of Lowry et al. (1951). RNA was then extracted by dissolving the pellet in 2 ml of 1M PCA and centrifuging as before. The supernatant was transferred to clean tubes for the determination of RNA. The pellet was rewashed and the supernatant combined with the RNA supernatant. The samples were frozen for the subsequent determination of total RNA by the method of Tsanev and Markov (1960). DNA was extracted by the addition of 5 ml of 2M PCA to each tube followed by incubation for 1 hour in a 70°C water bath. The protein fraction was re-pelleted by centrifugation for 20 minutes and the supernatant kept for DNA determination by the method of Munro and Fleck (1969)

B)Isolation and Measurement of L-[1-^{13}C] Leucine

The procedure used to isolate and measure L-[1-^{13}C] leucine content in muscle tissue was a modification of the technique described by Smith et al. (1988). The protein pellet obtained following protein/nucleic acid extraction was dissolved in 3 ml of 6M HCl, transferred to pyrex screw top boiling tubes, and hydrolyzed overnight (15-18 hours) at 120°C in an oven. The 6 M HCl was then evaporated to approximately 200 ul under nitrogen gas at 120-150°C using a Techne heating block and N_2 gas stream-evaporator (1 hour). The hydrolyzed protein was then applied to an ion-exchange column (Dowex 50w x-8, H^+ form, 100-200 mesh resin). The amino acids were eluted with 4 ml of 2M ammonium hydroxide after the column was washed with 3 ml of 1M HCl and 5-6 ml of doubly distilled water. The samples were dried in a rotary evaporator and then derivitized with 50-75 ul of N-methyl-t-butyldimethylsilyltrifluoro-acetamide (MTBSTFA) and an equal volume of pyridine in an oven at 85°C for 60-90 minutes.

C)Preparative Gas Chromatography(GC)/Isotope Ratio Mass Spectrometry

Preparative gas chromatography (GC), for the isolation of leucine, was done with a Pye Unicam 304 series chromatograph fitted with a post-column splitter (99:1 split ratio) and a wide bore glass column (6mm i.d. x 4.6m). The column was packed with 3% OV-101 on 80-100 mesh Chromosorb W HP (Phase Separations Ltd., Clwyd, UK). The derivitized amino

acid mixture in each muscle sample was injected into the injector port of the GC and the leucine fraction collected at the appropriate time. Based on leucine standards, the leucine fraction eluted as the third major chromatographic peak between 9-12 minutes. The leucine was collected from the post-column splitter in a home-made demountable glass U-trap cooled in liquid nitrogen. The temperature programme for the GC oven was 190°C for 1 minute, ramp at 4°C/minute to 210°C, hold 6 minutes, ramp to 290°C at 20°C hold 10 minutes to elute higher boiling compounds. The leucine collected in the U trap was removed by the addition of 0.5 ml of lithium citrate buffer, pH 2.2, to the trap followed by heating at 90°C for 30 minutes. The liquid was then transferred to a 20 ml Vacutainer tube and the U trap was rinsed with a further 0.5 ml of buffer. The rubber stoppers from the vacutainers were degassed overnight, in a sealed glass flask, in an oven under vacuum at 90°C. The samples were degassed at 140-150°C in a heating block for 30 minutes and placed on ice. Approximately 25 mg of ninhydrin was added to each tube on ice and the vacutainer was evacuated on a vacuum line. The ninhydrin reaction to liberate $^{13}CO_2$ and $^{12}CO_2$ was carried out in a 90°C water bath for 30 minutes. The tubes were then allowed to cool to room temperature and were filled with nitrogen. The $^{13}CO_2$ enrichment of the samples was determined by isotope-ratio mass spectrometry as described previously (Scrimgeour & Rennie,1988).

D)Plasma α-KIC Determination

Plasma α-KIC enrichment was determined by capillary gas chromatography/mass spectrometry (GC/MS) after derivitization to the quinoxalinol TFA derivative. Briefly, 4 ml of absolute ethanol was added to 500 ul of plasma and centrifuged at 4000 rpm for 10 minutes to precipitate proteins. The supernatant was transferred to clean tubes, evaporated under a gentle dry air stream overnight, and resuspended in 1 ml of distilled water. The ketone group of α-KIC was derivitized with 1 ml of 2% O-phenylenediamine (OPDA) at 100°C for 1 hour. The OPDA derivative was extracted in 2 x 2.5 ml washes of methylene chloride and the lower layer was pipetted into a clean tube. The OPDA derivative was then evaporated under a gentle stream of dry air. The final derivitization was performed with 75 ul BSTFA + 1% TMCS (Pierce Chemicals, Rockford, IL) and heating at 100°C for 1 hour.

The α-KIC enrichment of the plasma samples was determined with a VG-Trio-2 GC/MS (VG, Cheshire, England) with a Hewlett Packard Model 5890 GC (Hewlett Packard, USA). On - column injection of .3 ul of sample onto a 15 m fused silica column (0.25 mm i.d.)(DB5 J.W. Scientific, Rancho Verda, California) was performed and the m/z 232.2/233.2 ratios were monitored over a narrow mass range following electron impact ionization of the sample. The abundance of the ^{13}C enriched samples (233.2 amu) of α-KIC relative to the ^{12}C unenriched

species (232.2 amu) of α-KIC was taken from the listing of the mass spectrum acquired at the apex of the chromatographic peak. Details of the oven temperature programming and carrier gas are provided by Tarnopolsky et al. (1991). The inter and intra-assay coefficients of variation were <2 and 1% respectively.

E)Determination of Serum Creatine Kinase

Serum CK activity was measured with a kit (Sigma No 47-UV) and the values were expressed at 30°C. The increase in absorbance of NADH was monitored at 340 nm with a Unicam 1805 spectrophotometer. The intra and inter-assay coefficients of variation for a serum standard were 3.6% and 8.0% respectively.

2.2.4 Calculations

A)Dietary Intake of Leucine

The rate of appearance (Ra) of exogenous unlabelled leucine from the liquid meal appearing into the plasma was estimated at 583 umol leucine/g protein (Ensure, Ross Laboratories, PQ). This value was calculated based on a leucine content of 683 umol/g protein in Ensure and an assumed dietary absorption of protein of 85% (Cortiella et al.,1988).

B)Muscle Protein Synthetic Rate

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 [^{13}C] abundance in muscle protein obtained between the 2 biopsies from each arm, K_{Ep} is the mean plasma α-KIC enrichment for t=0 h, t=+2 h, and t=+4 h blood samples, and it is the incorporation time (in hours) between biopsy samples taken from the same arm (Nair et al.,1988).

C)Data Handling

Because of the small size of some biopsy samples, it was necessary to pool some control arm and exercise arm samples for the determination of protein, RNA, DNA, protein synthetic rate and RNA activity. This was valid because the muscle sample enrichments from the control and trained arms were similar for the first set of biopsies (after 2 hours of infusion). In addition, only single determinations of protein, total RNA, and DNA were made. Therefore the mean protein,total RNA, and DNA concentrations from the 2 biopsy samples for each arm were determined and the results are expressed in this manner. It was necessary to exclude the protein synthetic and RNA activity measures of subjects 1A and 3A respectively due to extremely small biopsy samples taken from the exercised arm at the end of the infusion protocol

2.2.5 Data Analysis

Descriptive data, elbow flexor strength and training protocol, leucine infusion parameters, and CK results were analyzed with a one-way analysis of variance. Protein,total RNA, and DNA concentrations were analyzed with a two-way

analysis of variance. Protein synthetic rates and RNA activity were analyzed with a two-way analysis of variance for unequal sample sizes (n=4 for group A; n=6 for group B). Pearson product correlations were determined to assess possible relationships between variables. A Tukey post hoc analysis was used when significant differences between means were obtained. A probability of $p<0.05$ was indicative of statistical significance. Values are expressed as means ± SD.

2.3 RESULTS

The two groups did not differ as to age, height, body weight, lean body weight, body fat, training history, and mean energy intake (Table I). Post-hoc analysis of dietary protein intake, however, showed that group B had a 32% greater mean protein intake than group A ($p<0.05$) (Table I). With reference to the biceps training session, the mean training intensity, training volume, and 1 RM for the three exercises was similar between groups (Table II). In addition, the mean infusion rate of L-[1-^{13}C] leucine, the mean dietary intake of leucine from the liquid meal, and the mean plasma α-KIC enrichments were similar between groups (Table III). The infusion time was slightly longer in group B than in group A ($p<0.05$) and the incorporation of [^{13}C] leucine was significantly elevated in the exercised compared to the control arm ($p<0.05$) but was not significantly different between groups (Table III).

The mean muscle protein contents and concentrations of total total RNA and DNA were similar between exercised and control arms and between groups (Table IV). A trend towards an increased RNA capacity was found in the exercised arm compared to the control arm ($p=0.058$). Protein synthetic rates were significantly elevated in the exercised biceps compared to the control biceps of groups A and B by 43% and 80% respectively (Table IV and Figure 1). The elevations in synthetic rates were statistically similar between groups. In addition, RNA activity was significantly greater in the exercised biceps compared to the control biceps of group A and group B by 25% and 89% respectively ($p<0.05$) (Table IV and Figure 2). The elevations in RNA activity were statistically similar between groups.

Post-exercise elbow flexor torque declined by 22% at 30°/s and 24% at 180°/s when compared to pre-exercise values ($p<0.001$) (Figure 3). In addition, absolute torque was significantly greater at 30°/s compared to 180°/s ($p<0.001$). A significant positive correlation was found between the percent change in elbow flexor torque measured at 30°/s and the percent change in protein synthetic rate between exercised and control arms ($r= -0.678$, $p<0.05$) (Figure 4).

Mean 22 hour post-exercise serum creatine kinase activity was elevated by 35% in group B (NS) (Table V) and no relationship was found between the the percent change in serum CK activity and the percent change in protein synthetic rate

between exercised and control arms (Figure 5).

A significant positive correlation was found between the subject's lean body mass and the percent change in protein synthetic rate between exercised and control arms ($r= 0.744$, $p<0.02$).

TABLE I. SUBJECT DESCRIPTIVE DATA

CHARACTERISTIC	GROUP A n=6		GROUP B n=6	
Age (years)	25.1 ± 3.9		23.1 ± 2.3	
Height (m)	1.76 ± 0.09		1.80 ± 0.05	
Body Weight (kg)	79.3 ± 5.1		83.0 ± 10.7	
Lean Body Weight (kg)	67.0 ± 5.1		73.3 ± 8.6	
Body Fat (%)	15.4 ± 5.1		11.4 ± 5.0	
Years of Training	4.9 ± 6.6		4.1 ± 3.3	
Energy Intake (kcal)	3076 ± 602		3196 ± 1057	
Protein Intake(g/kg/day)	1.88 ± 0.56	*	1.28 ± 0.35	

Values are means ± SD
* $p<0.05$

TABLE II. ELBOW FLEXOR STRENGTH AND TRAINING PROTOCOL

MEASURE	GROUP A	GROUP B
1 RM (kg)	21.6 ± 3.0	22.8 ± 5.0
Intensity (% of 1 RM)	70.7 ± 10.5	70.2 ± 10.9
Training Volume (kg)	524.1 ± 123.1	495.9 ± 133.6

Values are means ± SD

TABLE III. LEUCINE INFUSION PARAMETERS

SUBJECT	INFUSION RATE (umol/kg/hour)	DIETARY LEU	[^{13}C] KIC (APE)	INFUSION TIME (h)		[^{13}C] LEU ↑IN MUSCLE (APE)
1A	7.55	46.50	5.07	5.53	con	-----
					ex	-----
2A	9.10	63.99	6.24	4.75	con	0.0060
					ex	0.0109
3A	7.59	44.27	4.09	5.27	con	-----
					ex	-----
4A	9.10	41.66	5.68	4.77	con	0.0096
					ex	0.0104
5A	7.60	50.52	3.94	5.40	con	0.0104
					ex	0.0135
6A	7.21	39.50	4.21	6.70	con	0.0038
					ex	0.0153
x	8.02	47.74	4.87	5.40	con	0.0103
± SD	0.84	8.83	0.95	0.71		0.0038
					** ex	0.0163
						0.0094
1B	7.98	33.25	4.60	5.82	con	0.0060
					ex	0.0221
2B	7.55	39.41	4.74	6.37	con	0.0106
					ex	0.0196
3B	9.10	29.33	6.45	7.05	con	0.0106
					ex	0.0129
4B	7.41	54.74	4.04	6.57	con	0.0057
					ex	0.0188
5B	7.55	62.52	3.82	6.18	con	0.0072
					ex	0.0116
6B	7.55	53.58	4.10	6.30	con	0.0064
					ex	0.0115
x	7.86	45.47	4.63	6.38*	con	0.0081
± SD	0.64	13.33	0.96	0.41		0.0029
					** ex	0.0161
						0.0046

con=control arm ex=exercised arm APE= atom % excess
* p<0.05 group A vs group A ** p<0.05 exercised vs control arm

TABLE IV. PROTEIN/NUCLEIC ACID CONTENTS, PROTEIN SYNTHETIC RATES, AND RNA ACTIVITY

		PROTEIN CONTENT (% wet weight) n=6	RNA (ug/mg protein) n=6	DNA (ug/mg protein) n=6	PROTEIN SYN(ks)(%/hour) n=4	ks/RNA (ug pro/h /ug RNA) n=4
Group A						
con	$\bar{x}$	17.07	5.29	4.51	0.067	0.16
	±SD	10.74	1.95	1.82	0.0204	0.10
ex	$\bar{x}$	17.17	5.66	4.51	0.1007*	0.20*
	±SD	8.22	1.73	1.28	0.0330	0.09
Group B						
con	$\bar{x}$	15.42	5.33	4.90	0.0452	0.08
	±SD	5.35	0.86	1.38	0.0126	0.02
ex	$\bar{x}$	16.04	5.66	4.88	0.0944*	0.17*
	±SD	6.08	1.09	1.26	0.0363	0.05

Values are means ± SD
con=control arm ex=exercised arm
* significantly different from control arm $p<0.05$

TABLE V. SERUM CK ACTIVITY

SUBJECT	CK ACTIVITY (U/L) PRE EXERCISE	POST EXERCISE
1B	34.4	110.0
2B	123.0	143.5
3B	287.0	246.0
4B	82.0	102.5
5B	123.0	102.5
6B	41.0	226.0
$\bar{x}$	115.1	155.1
±SD	92.5	64.8

FIGURE 1. Biceps muscle protein synthetic rates in control versus exercised arms in groups A and B. Values are means ± SD.

* indicates a significant ($p<0.05$) difference between arms.

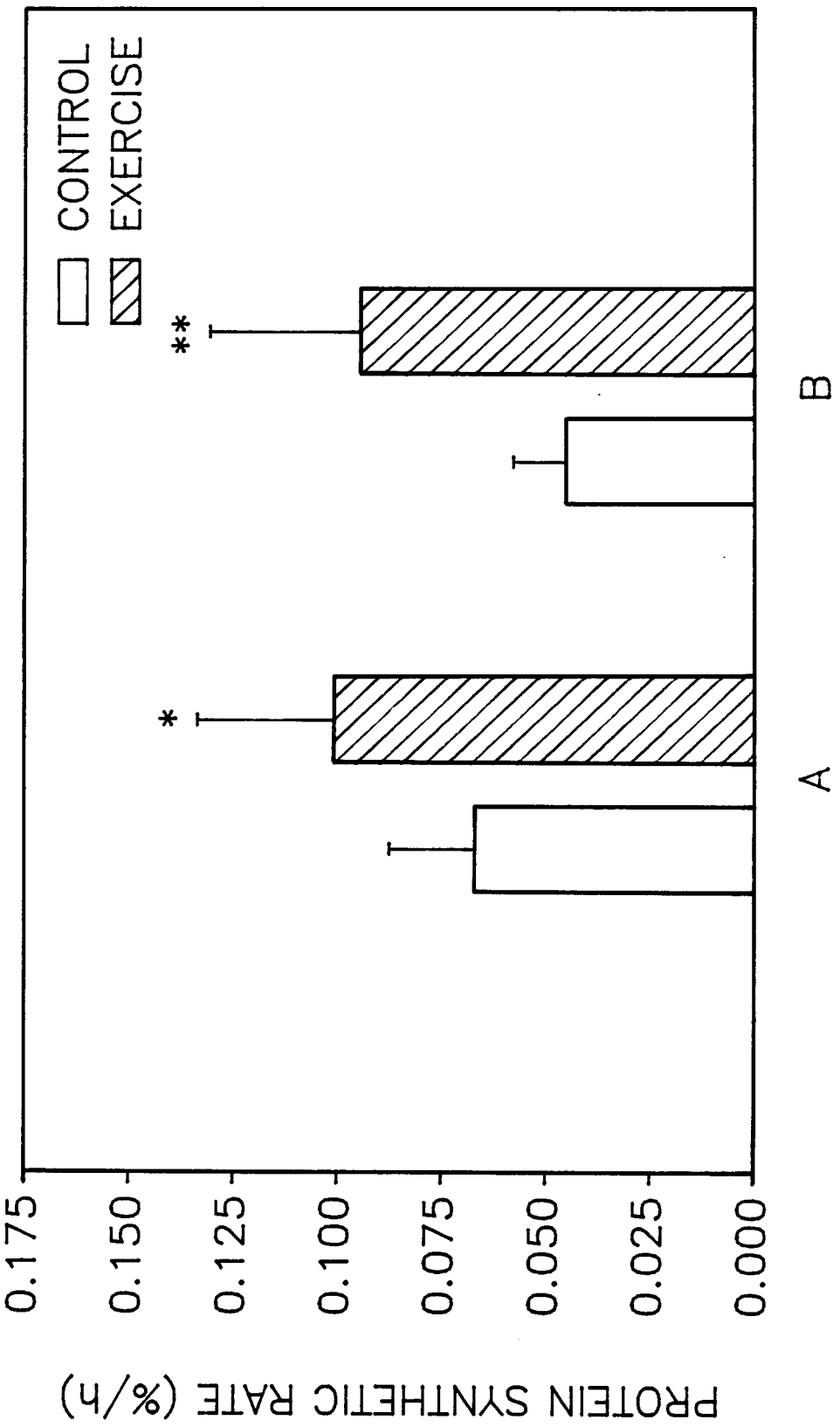

CONTROL
EXERCISE
PROTEIN SYNTHETIC RATE (%/h)
0.175
0.150
0.125
0.100
0.075
0.050
0.025
0.000
*
**
A
B

FIGURE 2. RNA activity of biceps from control and exercised arms of groups A and B. Values are means ± SD.

* indicates a significant ($p<0.05$) difference between arms.

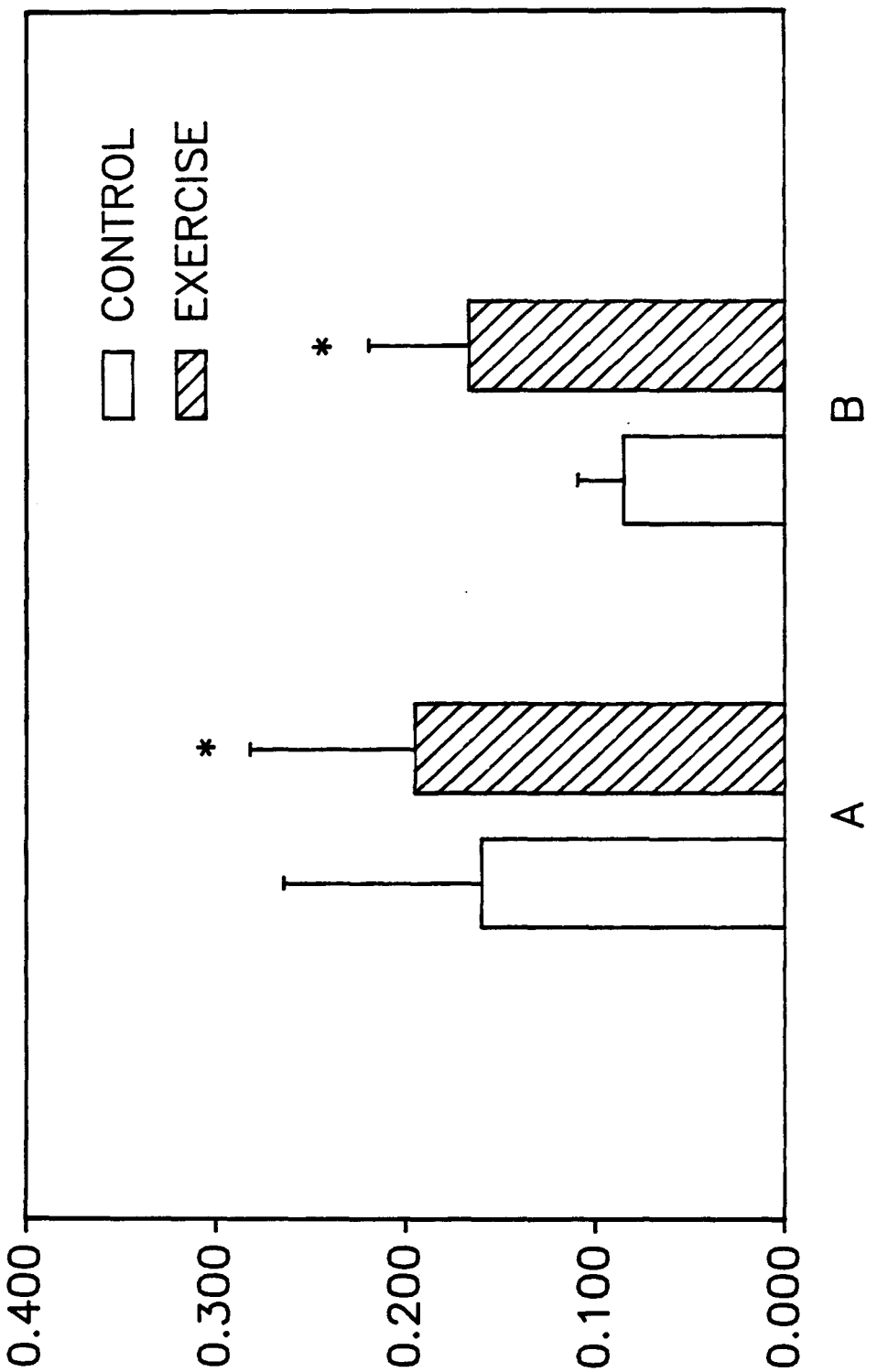

RNA ACTIVITY (ug pro/h/ug RNA)
0.400
0.300
0.200
0.100
0.000
CONTROL
EXERCISE
*
*
A
B

FIGURE 3. Elbow flexor torque of exercised arm from pooled data measured at 30°/s and 180°/s before and 15 minutes post-exercise. Values are means ± SD.

* indicates a significant ($p<0.001$) difference between pre to post-exercise torque.
** indicates a significant difference ($p<0.001$) between velocities.

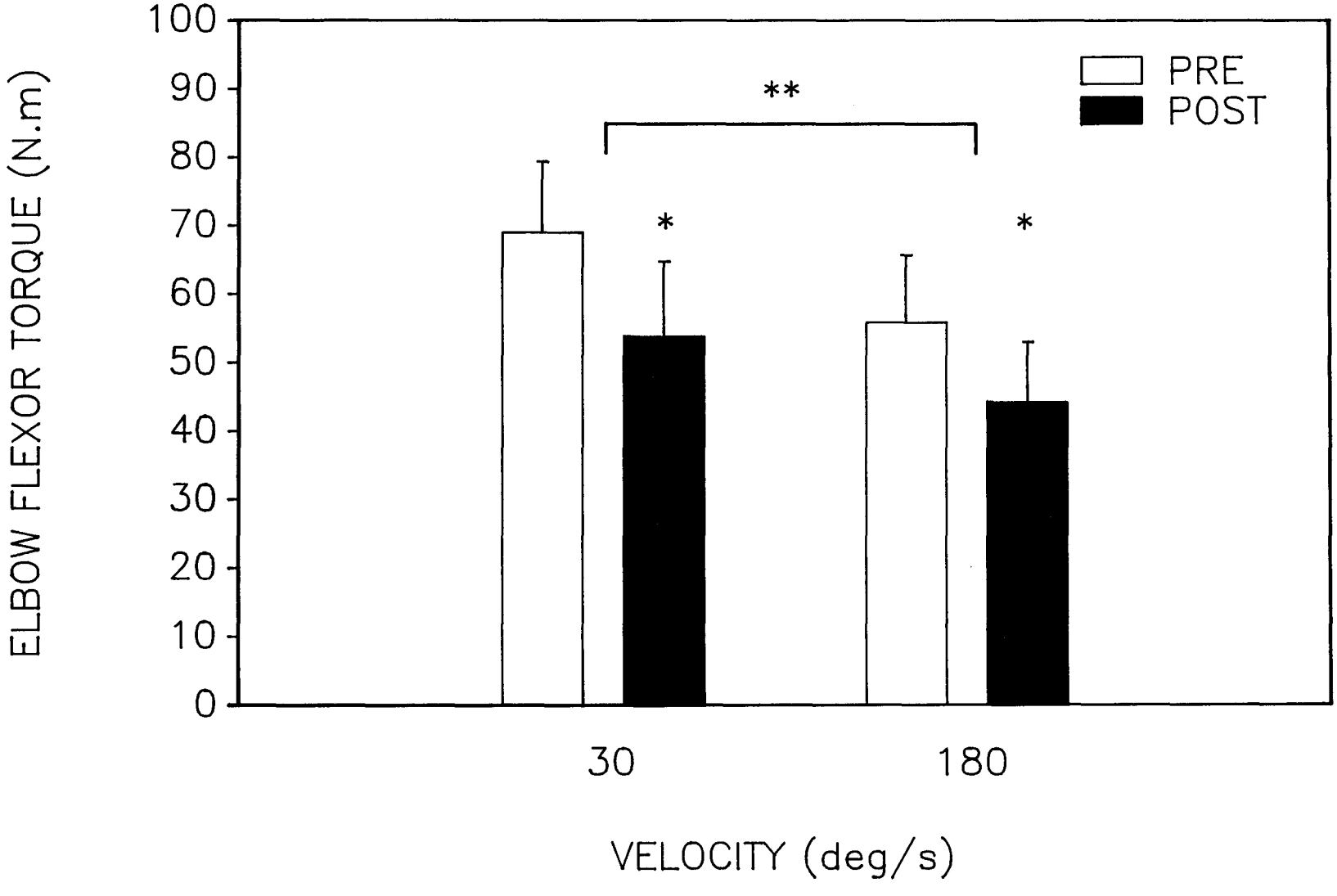

ELBOW FLEXOR TORQUE (N.m)
100
90
80
70
60
50
40
30
20
10
0
PRE
POST
**
*
*
30
180
VELOCITY (deg/s)

FIGURE 4. Relationship between percent change in elbow flexor torque measured at 30°/s and percent change in protein synthetic rate.

$r = -0.678$ ($p<0.05$) $R^2 = 0.459$

% change in synthetic rate= -4.879 x % change in torque - 2.985

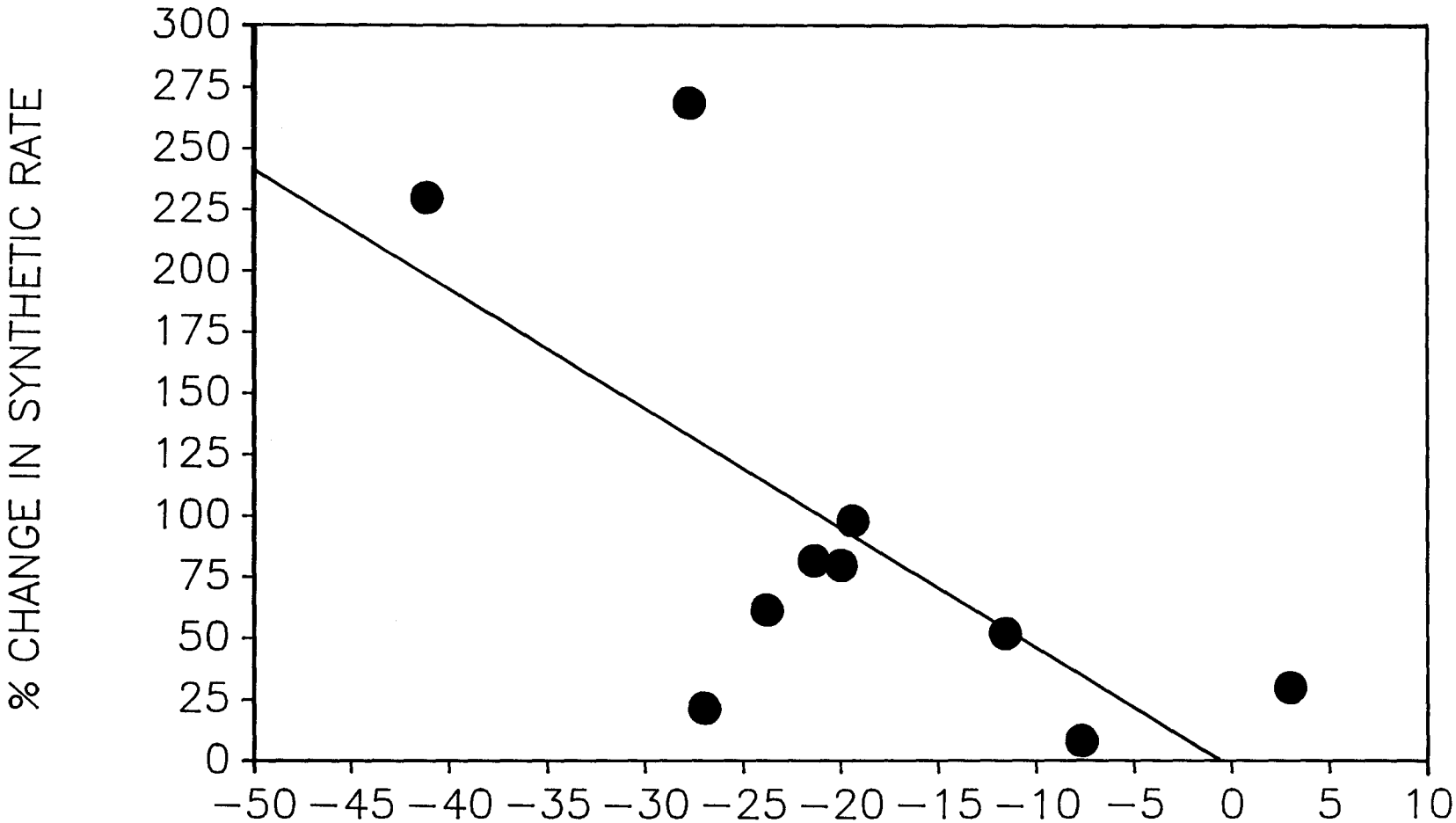

% CHANGE IN SYNTHETIC RATE
300
275
250
225
200
175
150
125
100
75
50
25
0
−50 −45 −40 −35 −30 −25 −20 −15 −10 −5 0 5 10
% CHANGE IN ELBOW FLEXOR TORQUE AT 30 deg/s

FIGURE 5. Relationship between percent change in serum CK activity and percent change in biceps protein synthetic rate.

r= 0.199 (NS) NS=not significant

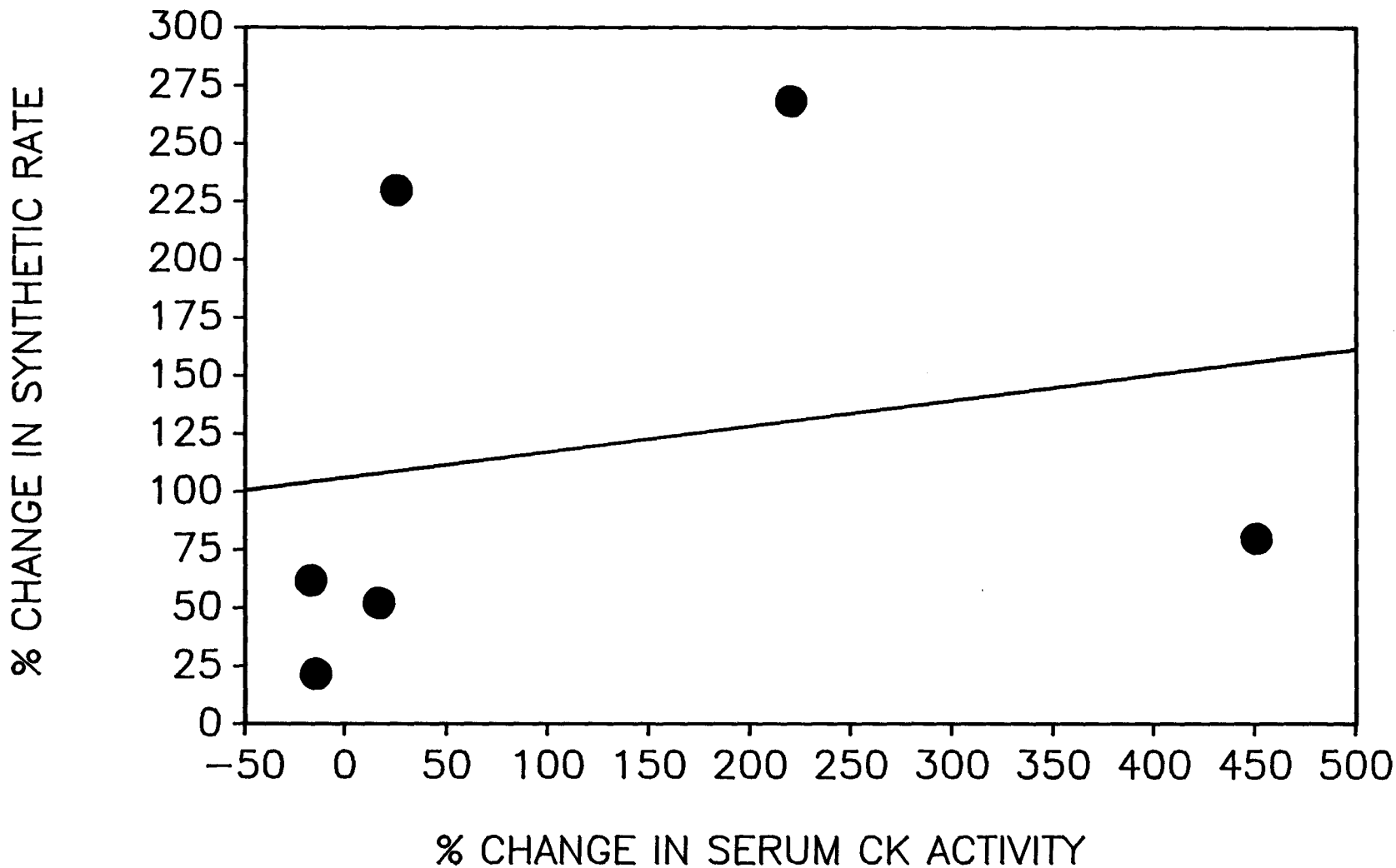
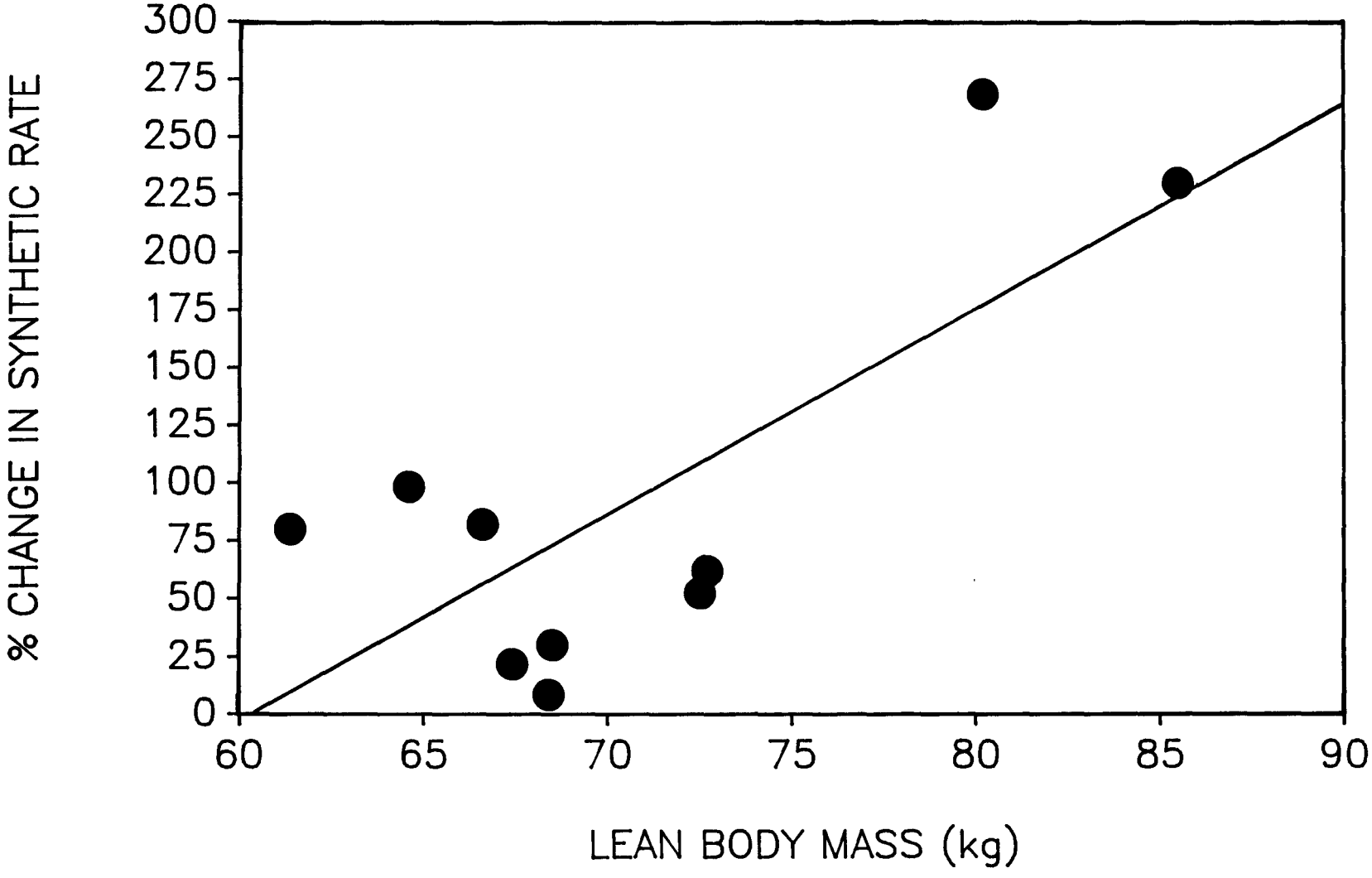

% CHANGE IN SYNTHETIC RATE
300
275
250
225
200
175
150
125
100
75
50
25
0
−50
0
50
100
150
200
250
300
350
400
450
500
% CHANGE IN SERUM CK ACTIVITY

FIGURE 6. Relationship between lean body mass (LBM) and percent change in biceps protein synthetic rate.

$r = 0.744$ ($p < 0.02$) $R^2 = 0.553$

% change in synthetic rate= 8.918 x LBM - 538.110


% CHANGE IN SYNTHETIC RATE
300
275
250
225
200
175
150
125
100
75
50
25
0
60
65
70
75
80
85
90
LEAN BODY MASS (kg)

2.4 DISCUSSION

The method used in this study to assess muscle protein synthesis requires muscle biopsies and rests on the assumption that post-exercise blood flow to the exercised and control arms is similar. The use of a small muscle group, such as biceps brachii, restricts the number of muscle biopsies which can be taken for acute/chronic determinations of protein synthesis. In addition, muscle protein synthetic rates reflect the contributions from soluble and myofibrillar proteins as well as from the different fiber types. In spite of these limitations, the use of the reciprocal pool model of leucine metabolism can provide valuable information on how mixed muscle protein responds to various physiological perturbations. This is because only two small biopsy samples are required from a given muscle for the calculation of a protein synthetic rate. In addition, myofibrillar protein accounts for approximately 70% of total muscle protein and thus makes the most significant contribution to muscle protein synthesis. A post-exercise difference in blood flow between exercised versus control arms could have affected the kinetics of labelled leucine uptake such that leucine was concentrated more in the exercised muscle. This is unlikely, however, since the leucine infusions were administered at times post-exercise when biceps blood flow in the exercised arm would have returned towards a value similar to that of the control biceps.

The major finding from this study was that an isolated bout of unilateral resistance training of the elbow flexors produced significant elevations in muscle protein synthesis in the exercised compared to the unexercised biceps. These results support the experimental hypothesis that acute post-exercise increases in muscle protein synthesis would occur following a single bout of resistance training. The relative difference in protein synthetic rates between exercised and control muscles was 33% for group A (4.4 h post-exercise) and 108% for group B (24.8 h post-exercise) and B. The relative differences in protein synthesis between exercised and control muscles were statistically similar between groups in spite of the higher synthetic rates found in group B than group A. Wong and Booth (1990a;1990b) reported acute elevations in muscle protein synthesis 12-17 h and 36-41 h following a concentric or an eccentric bout of resistance training performed by rats. The magnitude of the increase in muscle protein synthesis was dependent on the number of repetitions performed, resistance used, and type of muscle contraction. Acute elevations in muscle protein synthesis have also been documented following tenotomy and wing loading protocols (Golddberg et al.,1975;Laurent et al.,1978). The exercise protocol used in the present study was a potent stimulator of muscle protein synthesis since synthetic rates were elevated in the exercised biceps even after 24 hours following the training session. Future studies are required to more completely characterize

the time course of acute increases in muscle protein synthesis following resistance training.

Although subjects had not trained for the previous 3 days, the possibility that protein synthetic rates may have been acutely elevated in both arms from a previous training session cannot be dismissed. This appears unlikely, however, since the control biceps protein synthetic rates of 0.0670 %/h and 0.0452 %/h for groups A and B respectively are similar to values reported in sedentary fed humans (Smith & Rennie,1990).

An interesting observation was that the mean protein synthetic rates and RNA activities were 32% and 50% greater in the control biceps of group A compared to group B. A possible explanation for this finding was a 32% greater dietary protein intake in group A compared to group B. Dietary protein may exert a permissive effect in producing acute changes in muscle protein synthesis through the actions of insulin and plasma IGF-I levels (Millward & Rivers,1989). Because of the type of design used in this study, protein synthetic rates would likely have been equally affected by dietary protein intake in exercised and control arms within each group. However, the possibility exists that the between group differences in protein synthetic rates and RNA activity were due in part to dietary protein influences.

Muscle protein degradation was not assessed in this study and therefore a net rate of muscle growth could not be determined. However, a theoretical calculation of the net rate

of biceps muscle growth can be made to illustrate that muscle protein degradation must have increased in the exercised arm at some point following training. If one makes the assumptions that 1) rates of biceps muscle protein degradation are similar between control and trained arms at all times, 2) that net growth rates of 0.065%/h (based on table IV) are maintained for up to 24 hours post-exercise in the exercised arm and then quickly return to basal rates found in the control arm, and 3) the initial biceps mass is 250 grams, then the theoretical increase in biceps muscle mass would be 42 grams as a result of only 10 training sessions. This represents a relative increase in muscle mass of 16.8%. Relative increases in muscle cross-sectional area and fiber cross-sectional areas ranging from 10-30% have been documented only after several months of intense resistance training (MacDougall,1986; Tesch,1987). In addition, Yarasheski et al. (1990) reported chronic increases of 25% in quadriceps protein synthetic rates following 12 weeks of resistance training. The large acute elevations in muscle protein synthesis found in this study suggest that protein is synthesized in excess of the requirements for muscle growth. Therefore, it is clear from these observations that muscle protein degradation must have also been elevated in the exercised arm. Increases in protein degradation have been shown to accompany increases in protein synthesis in overloaded animal muscle (Goldberg et al.,1975; Goldspink et al.,1983; Laurent et al.,1978). In addition, urinary 3-

methylhistidine excretion, an index of myofibrillar protein degradation, has been shown to be chronically elevated in response to a resistance training program (Pivarnick et al.,1990). However, care must be taken in interpreting 3-MH data because of several assumptions about the method (Rennie & Millward,1983). Presently, there exists no valid technique, free of major assumptions, to assess the rate of protein degradation in muscle.

In order to assess whether gene transcription and/or translation was responsible for the increased rates of muscle protein synthesis, RNA capacity and RNA activity were determined in exercised and control arms respectively. RNA capacity is expressed as the total RNA content relative to non-collagenous protein content and has been used as an index of changes in transcription (Millward et al.,1973). RNA activity is expressed as the amount of protein synthesized per hour per unit of RNA and provides an index of the translational efficiency of the ribosomes (Waterlow et al.,1978) A trend towards an increased RNA capacity was found in the exercised compared to the control biceps of both groups. This suggested that there was an increase in total muscle RNA content. An alternate interpretation of selected decreases in rRNA and mRNA degradation in the exercised arm seems unlikely due to the long half-lives of these molecules (hours to days) (Waterlow et al.,1978) and the relatively short time course over which total RNA was measured in this

study. Since ribosomal RNA (rRNA) accounts for approximately 80% of total muscle RNA (Babij & Booth,1988; Waterlow et al.,1978), rRNA content may have increased in the exercised arms. However, changes in mRNA, if any occurred, could not be discerned from changes in rRNA because mRNA accounts for only 3-5% of total RNA (Waterlow et al.,1978). Wong and Booth (1990a;1990b) found no acute post-exercise changes in mRNA levels, coding for α-actin, in muscle that had been subjected to either a bout of concentric or eccentric resistance training.

A significantly higher RNA activity was found in the exercised biceps compared to the control biceps of both groups. The increases were 50% and 109% respectively for groups A and B relative to the control arm. RNA activity has been used as an index of translational efficiency of the ribosome (Millward et al.,1973; Waterlow et al.,1978). A change in translational efficiency has been shown to be the most sensitive mechanism for the acute regulation of muscle protein synthesis (Waterlow et al.,1978; Booth,1989). This is supported by findings in animal muscle undergoing compensatory hypertrophy. The RNA activity of these muscles is increased before there are any detectable changes in mRNA coding for specific muscle proteins (Laurent et al.,1978; Morrison et al.,1987). Gibson et al. (1987) found that decreases in muscle protein synthesis, in 5 week immobilized quadriceps, were associated with a 52% reduction in RNA activity. Similarly,

electrical stimulation of immobilized quadriceps, at an intensity corresponding to 5% of maximal voluntary contraction, was shown to increase muscle protein synthesis and RNA activity to a level comparable to the non-immobilized contralateral quadriceps (Gibson et al.,1988). The results from this study suggest that increases in RNA activity were the major cause of increases in muscle protein synthesis.

A number of possible candidates exist for stimulating changes in mRNA transcription and translation. Acute increases in the concentrations of hormones that stimulate anabolic processes in skeletal muscle have been documented following a single bout of resistance training (Kraemer,1988). Increases in plasma testosterone, growth hormone, insulin, and the insulin-like growth factors have been noted following a single weight training session (Guezennec et al.,1986; Kraemer et al., 1990; Vanhelder et al.,1984; Weiss et al.,1983). However, it is unlikely that testosterone, growth hormone or insulin play important roles in stimulating acute increases in muscle protein synthesis since: 1) all muscle groups (exercised and unexercised) are exposed to the same plasma hormone concentrations and yet hypertrophy only occurs in exercised muscle, 2) increases in plasma testosterone levels are short-lived and may be due to a decreased rate of clearance and not an increased rate of production (Kraemer,1988), 3) females are able to achieve the same relative amount of muscle hypertrophy

as males in spite of a 10 fold lower resting serum testosterone concentration (Cureton et al.,1989; Weiss et al,1983), and 4) the administration of insulin to healthy subjects does not appear to increase muscle protein synthesis but may inhibit muscle protein breakdown (Smith & Rennie, 1990). In addition, a recent study has demonstrated that growth hormone supplementation combined with a program of resistance training did not increase muscle hypertrophy above that found following resistance training only (Yarasheski et al.,1990). Chronic elevations in anabolic hormone levels have been found in elite weightlifters (Hakkinen et al.,1987; Hakkinen et al.,1988) and may play a role in maintaining a background anabolic environment in muscle.

A metabolic signal, generated by the hydrolysis of ATP and/or PCr, could possibly account for acute and chronic increases in muscle protein synthesis but the mechanism of how this might occur is unclear. A role for the prostaglandins, in stimulating acute and chronic changes in muscle protein synthesis, is possible but data regarding changes in $PGF_{2\alpha}$ levels in humans following resistance training is lacking. A positive relationship between $PGF_{2\alpha}$ levels and rates of muscle protein synthesis has been established (Reeds et al.,1987). In addition, a link between growth factor release and work-induced muscle hypertrophy has been found. Growth factors are small peptides that are synthesized in many cell types and stimulate a wide variety of cellular processes (Norman &

Litwack,1987). It is possible that a selective release of growth factors from the exercised biceps could have increased protein synthesis both acutely and chronically. Growth factor gene expression and release have been shown to increase in hypertrophying animal muscle (DeVol et al.,1990; Yamada et al.,1989). However, it is not known how growth factors would stimulate transcription and translation since they are classified as mitogens.

In order to assess possible contractile protein damage, maximal concentric elbow flexor torques were determined in the exercised arm at two different angular velocities immediately before and 15 minutes post-exercise. The maximal torque values declined by 22% at 30°/s and by 24% at 180°/s when compared to pre-exercise values. It is unlikely that metabolic factors such as hydrogen ion accumulation, lactate accumulation, or PCr depletion were responsible for the decline in maximal force generating capacity. Sahlin and Ren (1989) showed that maximal voluntary contraction force recovered by 4 minutes following the completion of a fatiguing isometric knee extension held at 66% of the maximal voluntary force. The 15 minute recovery period in this study can be considered to be sufficient for the regeneration of PCr stores. There is a possibility that muscle damage, caused by the training session, may have affected post-exercise torque output. Friden et al. (1983) noted large decreases in maximal leg extensor isokinetic torque measures for up to 6 days

following a bout of eccentric bicycle exercise. The largest decreases in torque occurred at the highest test velocities and morphological study of biopsy samples confirmed that type II muscle fibers displayed a greater incidence of Z-band streaming, broadening, and total disruption than type I fibers. Similarly, a force decrement of 60% was found for maximal isometric elbow flexion following the completion of 80 maximal eccentric elbow flexor contractions (Newham et al.,1987). The force decrements seen in this study were less than those reported by Newham et al. (1987) possibly because the subjects did not perform maximal eccentric contractions. The eccentric component of each contraction was limited by the subject's ability to lift the weight concentrically. In addition, if contractile protein damage damage is reflected by a decline in torque, then the results from this study suggest that damage occurred to both fiber types and was not selective for FT fibers.

A significant positive correlation was found between changes in elbow flexor torque performed at 30°/s and the percent change in muscle protein synthesis. This suggests that decrements in force, which may reflect contractile protein damage, could be associated with an increased protein synthetic rate. Friden (1983) noted the presence of polyribosome complexes close to points of focal muscle injury 3 days following a bout of eccentric exercise. In addition, myofiber damage may lead to the release of mitogens which

could stimulate satellite cell proliferation, differentiation, and migration to areas of injury (Bischoff,1986; Bischoff, 1986; Schultz et al.,1985).

Serum CK activity was measured in subjects from group B as a possible indicator of muscle damage. A 35% mean increase in post-exercise serum CK was found but was not statistically significant. This was possibly due to two subjects experiencing modest decreases in serum CK pre to post-exercise. In addition, it is possible that the high pre-exercise CK levels of these subjects was the result of a previous training session. The 3 day rest period, prior to the determination of pre-exercise CK, may have been insufficient in these subjects to allow serum CK levels to return to normal baseline. The time course for increases in serum CK can vary tremendously between subjects and can range from 1-7 days (Ebbeling & Clarkson,1989). Blood sampling at later time points might have resulted in a more pronounced CK elevation but an interaction between the damage produced by the muscle biopsies and the exercise session would have confounded an interpretation of the results. Finally, no relationship was found between the percent change in serum CK and the percent change in muscle protein synthesis.

There is no apparent physiological explanation to account for the positive correlation which was noted between lean body mass and the percent change in muscle protein

synthesis. It is possible that the relationship was spurious due to a clustering of the data points around two areas of the plot.

CONCLUSION

Changes in muscle protein synthesis were evaluated in two groups of resistance-trained subjects following a single bout of unilateral elbow flexor resistance training. Protein synthetic rates were elevated in the exercised biceps 4 and 24 hours post-exercise compared to the contralateral control biceps. The mechanisms that may contribute to these findings include an increase in RNA activity, a less pronounced increase in RNA capacity, and possible contractile protein damage, as assessed by a decrement in post-exercise maximal voluntary torque generating capacity and an increase in post-exercise serum CK levels.

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APPENDIX I: ANOVA SUMMARY TABLES

APPENDIX I-A

ANOVA SUMMARY FOR PROTEIN SYNTHETIC RATE

SUBJECT	SYNTHETIC RATE (%/hour)	
	EXERCISED ARM	CONTROL ARM
1A	0.0399	0.0725
2A	0.0687	0.0744
3A	0.0892	0.1158
4A	0.0707	0.1400
5A	0.0405	0.1492
6A	0.0677	0.1029
1B	0.0362	0.0440
2B	0.0345	0.1138
3B	0.0524	0.0844
4B	0.0402	0.0723
5B		
6B		

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	.000	1	.001	1.000	
ERROR	.007	8	.001		
WITHIN BLOCKS/SUBJECTS					
ARM	.008	1	.008	8.000	.021
GROUP X ARM	2.93 -04	1	.000		
ERROR	.005	8	.001		

APPENDIX I-B

ANOVA SUMMARY FOR RNA ACTIVITY

SUBJECT	RNA ACTIVITY (ug protein synthesized/hour/ug RNA) EXERCISED ARM	CONTROL ARM
1A	.12	.07
2A	.12	.12
3A	.26	.31
4A	.28	.14
5A	.22	.06
6A	.18	.13
1B	.10	.08
2B	.23	.07
3B	.12	.09
4B	.15	.08
5B		
6B		

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	.013	1	.013	1.857	.208
ERROR	.052	8	.007		
WITHIN BLOCKS/SUBJECTS					
ARM	.016	1	.016	8.000	.021
GROUP X ARM	.003	1	.003	1.500	.254
ERROR	.020	8	.002		

APPENDIX II: ETHICS APPROVAL/SAMPLE CONSENT FORMS

McMASTER UNIVERSITY
HAMILTON, ONTARIO, CANADA

COMMITTEE ON
THE ETHICS OF RESEARCH ON HUMAN SUBJECTS

TO: The Office of Research Services

RE: Dr. J.D. MacDougall

TITLE: The time course for increased skeletal muscle protein synthesis following unilateral arm strength training

The above named applicant has submitted an application to the Committee on Ethics of Research on Human Subjects.

The Committee has reviewed this request and finds that it meets our criteria of acceptability on ethical grounds. The review has been conducted with a view toward insuring that the rights and privacy of the subject have been adequately protected; that the risks of the investigation do not outweigh the anticipated gain; and that informed consent will be appropriately obtained.

We concur in all necessary endorsements of the application.

S. Martin Taylor

Date: 19 June 1990

For the Committee on the Ethics of Research on Human Subjects

C.K. Bart, Associate Professor, Business
T. Beckett, Judge, Unified Family Court
B. Donst, Ecumenical Chaplain, Chaplains' Office
D. Elliott, Associate Professor, Physical Education and Athletics
J. Gaa, Associate Professor, Business
T. Kroeker, Lecturer, Religious Studies
R. Milner, Associate Professor, Clinical Epidemiology and Biostatistics
R.J. Preston, Professor, Anthropology
J. Synge, Associate Professor, Sociology
S.M. Taylor, Professor, Geography (Chairman)

McMASTER UNIVERSITY
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THE TIME COURSE FOR INCREASED SKELETAL MUSCLE PROTEIN SYNTHESIS FOLLOWING UNILATERAL ARM STRENGTH TRAINING.

INFORMATION AND CONSENT FORM

The principal investigators for this project are Dr. Duncan MacDougall and Alan Chesley. 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 subject for this study.

A. PURPOSE

The purpose of this study will be to examine the effects of a single session of arm training(with weights)on muscle protein synthesis 4 or 24 hours following exercise.

B. PROCEDURES

During your first visit to the lab your maximal arm strength will be assessed for three different weight lifting exercises. In addition, your percent body fat will be determined by underwater weighing.

During your second visit to the lab you will be asked to perform the same three weight lifting exercises that you did during your first visit. Immediately after or 20 hours following exercise you will receive an injection of a small amount of the 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 6 hours.

Following this, a total of four muscle samples will be taken from the biceps of both arms(2 per arm) by what is known as a needle biopsy procedure. This will be done either 4 or 24 hours after exercise. The biopsies involve the removal of a small amount of muscle tissue(50-100 mg) by a skilled physician with a sterile hollow needle under local anaesthesia.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. Finally, blood samples will be drawn from a hand /arm vein periodically throughout the study.

The amount of the labelled protein which appears in your exercised arm compared with your non-exercised arm allows us to determine how effective the intervening exercise bout was in stimulating amino acid uptake and muscle protein synthesis.

C. DETAILS OF THE PROCEDURES AND THEIR POSSIBLE RISKS

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. Under a local anaesthetic 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 6 hours using a Harvard infusion pump. There is no discomfort associated with this(in fact you will be unaware that anything is happening) so bring a book to read over this time.

This infusion 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 3 days. Since the injected tracer is not radioactive there are no known risks to health/fertility with over 30 years experience of similar use of stable isotopes in patients.

Needle Biopsy Procedure

This procedure involves the local injection of an anaesthetic(freezing)into the skin of the biceps area, after which a small(4mm) incision will be made and a small (50-100 mg) piece of muscle will be removed with a special needle. After the procedure a suture will close the skin and pressure will be applied to minimize bruising. Most people report little discomfort with the procedure. It will be performed by a physician who is familiar with the technique.

Complications with the procedure are rare. However, in our experience with athletes, less than 1 in 400-500 subjects experience a local skin infection, 1 in 30-40 have a temporary(up to 4 months) localized loss of sensation in the skin at the site 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 $100 for the completion of the study to help compensate you 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 in 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 Witness

_____

Date