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EXERCISE-RELATED CHANGES IN PROTEIN TURNOVER IN MAMMALIAN STRIATED MUSCLE

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Summary

Contractile activity is an important determinant of the size, rate of protein turnover and phenotypic properties of muscle. Animal models that decrease muscle activity invariably accelerate the rate of protein degradation, usually complementing decreases in the rate of protein synthesis. The net effect is muscle atrophy. By contrast, increased activity and/or passive stretch enhance the synthesis of new proteins, whilst protein catabolism may be either decreased or increased. Muscle hypertrophy results.

Endurance activities in man and animals usually induce cardiac hypertrophy, and increased fatigue resistance in skeletal muscle. During exercise the whole body and its skeletal musculature exhibit a negative nitrogen balance, and there is general agreement that rates of protein synthesis are decreased. Changes in protein degradation are, however, much less clearly defined.

Resistance exercises induce the opposite changes, with the size of the heart remaining unchanged whilst the bulk and strength of skeletal muscle increase. No real consensus currently exists about the nature of the changes in protein turnover with this type of exercise. More carefully designed and executed experiments are required.

Introduction

Exercise induces changes in the overall size and/or metabolic and contractile characteristics of striated muscles; the precise nature of the adaptations is determined by the type of exercise undertaken.

A link between the adaptive growth of striated muscle and exercise was recognised as long ago as the middle of the 18th century. It was noted that animals that had been subjected to heavy physical exertions possessed an increased muscle mass and larger hearts. Although a great deal of progress has been made since those early observations, the complexity of this subject precludes making simple yet meaningful descriptions of the adaptations that occur in response to exercise. This is perhaps not surprising when one considers the number of variables involved in the different exercise regimes, e.g. differing in their frequency,

Key words: endurance exercise, resistance exercise, muscle activity, protein synthesis, protein degradation, muscle fibre types, oxidative capacity.

duration and intensity, and in the age and sex of the subjects and the extent of their prior training. Many of the animal experiments performed with the intention of mimicking the physical activity performed by humans are inappropriate and the results potentially misleading if taken out of context (see Booth and Thomason, 1991).

The target tissues, e.g. skeletal muscle, are also complex and heterogeneous with respect to their cell types. Even after excluding the non-contractile elements (20–25 %), skeletal muscles are composed of at least three histochemically distinct fibre types, as stained for the myosin ATPase. Slow oxidative (SO or type I), fast oxidative glycolytic (FOG or type IIA) and fast glycolytic (FG or type IIB) fibres can be detected in most muscles (Saltin and Gollnick, 1983). Usually one of these fibre types predominates, thereby bestowing on that muscle its metabolic and fast- or slow-twitch contractile properties, characteristics that vary as a function of age as well as in response to exercise. The three fibre types are known to respond to differing extents, depending upon the type of exercise undertaken. Indeed, endurance activities can cause some of these fibres to convert from one type to another, e.g. fast to slow, thereby altering the phenotypic properties of the muscle as a whole.

It is generally accepted that in performing any muscular activity SO fibres are recruited first. Additional requirements to speed up movements, or to generate more force, will be accompanied by progressive increments in the activation of FOG and FG fibres. So, in high-intensity resistance exercises, which demand rapid and high force output (e.g. weightlifting and athletic throwing events), the appropriate muscles become larger through an appreciable hypertrophy of all fibres, i.e. SO, FOG and FG fibres (Goldspink, 1980). Whilst the hypertrophy is usually greatest in the faster fibres, all increases in fibre diameters can be attributed to a greater accumulation of myofibrils. In contrast to these adaptations in skeletal muscle, dynamic exercises do not appreciably alter the size of the heart.

Endurance exercises (e.g. long-distance running) usually consist of highly repetitive forms of activity against small loads. Such repetitive usage causes significant changes in the metabolic status of skeletal muscles, without dramatically changing their overall size. Although slow fibres may undergo some hypertrophy, the simultaneous atrophy of some fast fibres leaves the muscle's size and power output relatively unchanged (Goldspink, 1980). The primary response to endurance exercise is to enhance the fatigue-resistance of skeletal muscle. In contrast, endurance activities in animals and man cause the heart to enlarge by approximately 10–30 %. It is thought that this hypertrophy represents increases in the length and diameter of the individual muscle cells, thereby causing the longitudinal and transverse diameters of both ventricles to increase. A slower heart rate and greater stroke volume, at rest and during any given workload, develop after a fairly limited period of vigorous training; changes that appear roughly to parallel the increases in heart size (Buuck and Tharp, 1971).

These different adaptive responses to resistance and endurance exercises will be discussed in more detail below.

Techniques for measuring protein turnover in striated muscles

Muscle growth (i.e. protein accumulation) and atrophy (i.e. net loss of protein) occur as a result of changes in the relative rates of protein synthesis and protein degradation; both regulatory processes being under physiological control.

Protein synthesis

The biochemical events and regulatory mechanisms involved in the synthesis of proteins are generally well understood (Pain, 1978). The most reliable technique for measuring protein synthesis rates *in vivo* in animals and man is the 'flooding dose' method developed by McNurlan *et al.* (1979). This method enables synthesis rates in most tissues to be measured over short periods, e.g. 10 min in animals. This is important as it eliminates problems such as (i) time-related changes in the specific radioactivity of the amino acid precursor pool(s), and (ii) amino acid re-utilisation, which can vary with changes in the rate of protein degradation within the body's tissues. This technique is designed to expand, or flood, the tissue's amino acid precursor pool(s) so that its specific radioactivity becomes approximately equivalent to that of the plasma, thereby minimising difficulties in determining whether the pool is intracellular or extracellular in location (Waterlow *et al.* 1978). Constant infusions of stable isotopes (e.g. [^{13}C]leucine) are still given in man because of the large expense involved in applying the 'flooding dose' method on a body weight basis.

Synthesis rates can be altered by changing the rates of transcription and/or turnover of mRNA (i.e. pre-translational events), in association with the availability of ribosomes to translate the message into the encoded proteins. In addition, translation itself can be modified by changing the rates of peptide initiation, elongation or termination (Pain, 1978). In these ways the efficiency with which the mRNA is read can be varied.

Protein degradation

Muscle proteins are in a continuous state of turnover: the average half-lives in rat adult slow- and fast-twitch muscles are 12.1 and 18.3 days, respectively (Lewis *et al.* 1984). By contrast to the sophisticated understanding surrounding protein synthesis, our knowledge of the sequential steps and control mechanisms involved in the degradation of intracellular proteins is rudimentary. Considerable progress has been made in recent years. Nonetheless, we are still far from the ultimate goal of describing the coordinated actions of peptide hydrolases in the degradation of muscle proteins.

A wide range of endo- and exopeptides has been found in the lysosomes of many tissues, thereby endowing this subcellular organelle with the potential for degrading most, if not all, of the cell's protein. At various times, both cathepsins D and B have been regarded as the major proteolytic activity within lysosomes. However, lysosomes are seldom seen when muscles are examined microscopically, and the need to apply harsh mechanical measures to disrupt the connective tissue

and myofibrils has frustrated attempts to isolate such lysosomal particles unequivocally.

Despite its undoubted potential for hydrolysing proteins, the precise role of the lysosome in the normal turnover of cellular proteins remains unclear. The dissembly and partial, or complete, degradation of internal structures, e.g. the myofibrils, may be associated with proteolytic activities optimal at neutral or alkaline pH. Ca^{2+}-activated enzymes, which have the ability to remove the Z-lines from washed myofibrils, are among the small group of enzymes believed to be cytosolic in origin (Busch *et al.* 1972). Amino- and dipeptidases have also been described, along with a casein-degrading enzyme (pH 8.3) that is thought to be associated with the myofibrils (Pennington, 1977). Only in a few instances have such peptide hydrolases been shown to be active against muscle protein substrates; e.g. the Ca^{2+}-activated neutral proteinase is capable of degrading tropomyosin and troponins T and I, but not other myofibril proteins such as myosin, actin, α-actin and troponin C (Dayton *et al.* 1975). Most other enzymes, however, have been assayed against synthetic substrates. Hence, considerable progress in this area is still required if we are to unravel the complexities surrounding the degradation of intracellular proteins.

Induced changes in the activities of cathepsins B and D appear to parallel alterations in the degradative rates of muscle proteins, both as a function of age and in various experimental systems (Goldspink and Lewis, 1987). Although these observations point to a link between lysosomes and normal protein turnover, it is difficult to envisage how such a membrane-bound system is able to discriminate between, and thereby explain, the widely differing half-lives of individual proteins and structures such as the myofibrils in muscle.

A major problem in attempting to study protein degradation in the body's tissues is the lack of availability of a reliable method for measuring protein breakdown *in vivo*. The non-exponential decay of pre-labelled proteins and the efficient reutilisation of amino acids make it difficult to obtain meaningful rates *in vivo* (Waterlow *et al.* 1978). Alternative approaches include calculating the rates of protein degradation by subtracting the measured rates of growth from those of protein synthesis (Garlick *et al.* 1979; Lewis *et al.* 1984). Such indirect rates of degradation are generally in good agreement with changes in the activities of certain proteinases, e.g. cathepsins B and D (Goldspink and Lewis, 1987). Alternatively, small muscles can be incubated *in vitro* in the presence of an inhibitor of protein synthesis, e.g. cycloheximide, and the release of tyrosine or phenylalanine into the medium measured to evaluate protein breakdown (e.g. Fulks *et al.* 1975). However, even small thin muscles have been shown to develop an anoxic core within the central fibres (Maltin and Harris, 1985), casting doubt on the viability of such tissue preparations. These isolated muscles also exhibit a negative nitrogen balance (Fulks *et al.* 1975; Goldspink *et al.* 1983). The relative merits and shortcomings of these techniques have been discussed by Garlick *et al.* (1979).

For more extensive information and discussion on the techniques available for

measuring protein turnover in the tissues of man and animals, the reviews of Waterlow *et al.* (1978), Zak *et al.* (1979), Khairallah *et al.* (1985), Glaumann and Ballard (1987) and McNurlan and Garlick (1989) are recommended.

Contractile activity and muscle size

Developmental changes

In the mammalian foetus, skeletal muscle contributes approximately 15–20 % to the whole-body protein mass and its total rate of protein synthesis (Goldspink *et al.* 1985). In contrast, in the neonate and adult, the musculature increases dramatically to make the largest single contribution to whole-body protein (50–75 %) and its rate of synthesis (20–40 %). Undoubtedly the rapid postnatal growth of this tissue is related, at least in part, to the increased activity of the animal, i.e. its increasing demands for locomotion, weightbearing and maintenance of body posture.

Postnatal differentiation into adult fast- and slow-twitch muscles is also dependent upon the activity pattern imposed on the muscles. This may be due to either the frequency of discharge in the motor neurones, the aggregate number of impulses delivered to, or the amount of activity imposed on, the muscle (Pette and Vbrova, 1985). The latter two physical parameters are currently considered to be more important than frequency *per se*, with slow postural muscles receiving more impulses per unit time and, consequently, performing continuous work. By changing the activity pattern of the muscle, even fully differentiated muscles can be transformed from one type to another (see below).

During postnatal life the rapid growth of muscle is accompanied by increased total rates of protein synthesis, i.e. the amount of protein synthesised per day in the whole muscle, with the share of the contractile proteins being 50–60 % of the total (Waterlow *et al.* 1978; Lewis *et al.* 1984). However, when expressed as fractional rates of synthesis or degradation (i.e. the percentage of the protein mass either synthesised or degraded per day) such rates are independent of muscle size. With increasing age, both fractional rates progressively decline (Table 1). The large imbalances between the two rates, in favour of synthesis, in the perinatal period gradually diminish until non-growing conditions are reached in the muscles of the adult (Table 1). These developmental changes in protein turnover apply to sarcoplasmic and myofibrillar proteins alike, and appear to be partially linked to the changing state of differentiation in the muscles. However, throughout development the absolute rates of protein synthesis and degradation correlate with the level of activity expressed in the muscles. Hence, age for age, continuously active muscles, such as the heart and soleus, share similarly high rates of protein turnover, which are consistently greater than those measured in the less frequently activated fast-twitch muscles (Table 1).

Like the metabolic rate, the rates of protein synthesis and protein degradation in muscles of adult rats (ie. 3.0–6.5 % per day; Table 1) are considerably higher than those measured in the muscles of man at 1–2 % per day (Gibson *et al.* 1987).

Table 1. *Age-related changes in the fractional rates of protein synthesis and degradation in four striated muscles of the rat*

		Fractional rate of protein synthesis (% day^{-1})				
		Foetal age (days)		Postnatal age (weeks)		
Muscle		18	20	3	8	105
Anterior tibialis	FRS	–	2.1±0.3	12±0.3	9.1±0.4	3.8±0.4
	FRB	–	–	5.9	7.3	2.8
Gastrocnemius	FRS	–	65±9.1	16±0.6	10±0.3	3.4±0.3
	FRB	–	30	5.2	8.5	5.6
Soleus	FRS	–	–	19±1.1	15±0.7	5.7±0.2
	FRB	–	–	8.2	11.6	5.1
Cardiac	FRS	74±1.3	47±3.8	19±0.4	12±0.4	6.6±0.3
	FRB	33	25	15	9.8	6.0

Adapted from Lewis *et al.* (1984) and Kelly *et al.* (1984).

Fractional rates of protein synthesis (FRS) were measured *in vivo* by the 'flooding dose' method of McNurlan *et al.* (1979) and are expressed as percentage per day. These rates are presented as means+S.E.M. Indirect rates of protein breakdown (FRB) were calculated from the difference between the measured rates of muscle growth and protein synthesis (Lewis *et al.* 1984; Kelly *et al.* 1984), and S.E.M. values cannot be provided with these values. Nonetheless, these developmental changes in protein degradation correlate with the age-related changes in the specific activities of cathepsins B and D (Goldspink and Lewis, 1987).

The anterior tibialis and gastrocnemius are predominantly fast-twitch muscles and the soleus is a slow-twitch muscle in the rat.

Experimental models altering muscle activity

Decreased activity

When muscles are rendered less active (Hnik *et al.* 1985), as a consequence of weightlessness, denervation or immobilisation in a shortened position, they undergo atrophy through decreases in both their cross-sectional area and myofibril length. Under such conditions of inactivity the average rates of protein synthesis are decreased and the rates of protein degradation increased (Table 2). The synthesis of α-actin has also been found to be decreased 6 h after inducing inactivity (Watson *et al.* 1984). The resultant effects of disuse are more dramatically expressed in the postural muscles (Loughna *et al.* 1986; Goldspink *et al.* 1986), with reductions in the number of SO fibres and phosphocreatine concentrations. 40 and 20 % decreases in the concentration of mRNA and the synthesis rate of cytochrome *c*, respectively, are changes consistent with a fall in the oxidative capacity (Morrison *et al.* 1987).

Accelerated rates of protein degradation are also found in muscles rendered less active as a consequence of tenotomy. Indeed, increased protein catabolism is a consistent feature of all experimental models that decrease muscle activity (Table 2).

The return of activity to previously inactive muscles rapidly increases the

ble 2. *Changes in skeletal muscle size and protein turnover in a variety of animals and man in response to altered muscle activity*

Experimental procedure	Muscle studied	Induced changes in Protein synthesis	Protein degradation	Muscle size	References
Denervation	EDL[a], soleus[a] diaphragm[b]	+ or −*	+	−	1–4, 13, 20
Simulated weightlessness	EDL[b], soleus[b]	−	+	−	6, 7, 16
Immobilization without stretch	EDL[a,b], soleus[a,b]	−	+	−	5–8, 17
Restoration of activity following immobilization	EDL[a], soleus[a], AT[a], plantaris[a], quadriceps[b]	+	−	+	9, 18
Immobilization under stretch (*in vivo*)	EDL[a,b], soleus[a,b], ALD[b], PLD[b]	+	+	+	5–7, 10, 12
Stretched (*in vitro*)	EDL[a]	+	−	+	11
Tenotomy	Gastrocnemius	0	+	−	13
Tenotomy of synergist	(i) Soleus[a,b,c]	+	+	+	13, 14
	(ii) Soleus[b]	+	−	+	15
Stretch+electrical stimulation	EDL[b], AT[b]	+	+	+	19

* Dependent upon the timing after nerve section.

Increases (+), decreases (−) or no changes (0) in the rates of protein synthesis or protein degradation in the muscles specified were measured either [a]*in vitro* (e.g. Fulks *et al.* 1975), [b]*in vivo* (e.g. McNurlan *et al.* 1979) or [c]in muscle homogenates.

Abbreviations for the muscles are the extensor digitorum longus (EDL), anterior tibialis (AT) and the anterior (ALD) and posterior (PLD) latissimus dorsi.

References: 1, Goldberg (1969*a*); 2, Goldspink (1975); 3, Goldspink (1978); 4, Turner and Garlick (1974); 5, Goldspink (1977*a*); 6, Goldspink *et al.* (1986); 7, Loughna *et al.* (1986); 8, Booth and Seider (1979); 9, Goldspink (1977*b*); 10, Laurent *et al.* (1978); 11, Kameyama and Etlinger (1979); 12, Gregory *et al.* (1990); 13, Goldspink *et al.* (1983); 14, Hamosh *et al.* (1967); 15, Goldberg (1969*b*); 16, Jasper and Tischler (1984); 17, Gibson *et al.* (1987); 18, Gibson *et al.* (1988); 19, Goldspink *et al.* (1991); 20, Furuno *et al.* (1990).

average rates of protein synthesis, whilst restoring the previously elevated rates of protein degradation to normal (Table 2). Cytochrome *c* mRNA concentration and synthesis rates are also increased by 25 and 90 %, respectively, with the restoration of normal activity (Morrison *et al.* 1987). Hence, changes in the rates of both transcription and translation appear to be involved in the adaptive responses in the mitochondria. In continuously active postural muscles, the pattern of usage seems to be important in maintaining muscle size, mitochondrial density, the higher rates of protein turnover (Table 1) and the slow contractile characteristics (Lomo *et al.* 1980). It seems that the developmental programming of muscles is to be 'fast' unless high levels of contractility are imposed on the muscle. Only then are the 'slow' genes of multi-gene families, e.g. slow myosin heavy and light chains

expressed. If postural muscles are rendered less active, they revert back to expressing 'fast genes', with consequent changes in contractile characteristics (Goldspink *et al.* 1991). Greater activity levels also appear to make both skeletal and cardiac muscle less responsive to catabolic stimuli such as glucocorticoid hormones and starvation (McNurlan and Garlick, 1989; Kettelhut *et al.* 1988). It has been suggested that under such conditions any obligatory loss of muscle protein, to serve as amino acid substrates for gluconeogenesis, can more effectively be spared from the fast, less frequently used muscles.

The importance of muscle length

Contractile activity is not the only mechanical determinant of muscle size. Muscle length and stretch are also important. When muscles are immobilised in a lengthened and stretched position they increase their cross-sectional area and number of sarcomeres in series; the latter is an adaptation designed to restore optimal overlap between the myosin and actin (Williams and Goldspink, 1971; Williams *et al.* 1986). Despite the presumed activation of the stretch reflex, chronic electromyographically recorded activity in these stretched muscles actually fell to two-thirds of that measured in normal controls (Hnik *et al.* 1985). In keeping with the reduced activity in these muscles, the rate of protein degradation increased (Table 2). At the same time, stretch greatly enhanced protein synthesis such that the net effect was muscle hypertrophy (Table 2). Similar observations have been made on mixed and myofibrillar protein synthesis and breakdown in the anterior (ALD) and posterior (PLD) latissimus dorsi muscles of the chicken when stretched by attaching weights to the wing (Table 2).

As similar changes to those described above are found in denervated muscles when stretched (i.e. in the absence of any stretch reflex), the responses must be predominantly myogenic (Goldspink *et al.* 1974; Goldspink, 1978).

If the soleus muscle is rendered 90–95 % less active than its control (Hnik *et al.* 1985), by immobilizing the ankle in dorsal flexion, protein synthesis decreases by 60 % and a 35 % loss of protein occurs in just 3 days (Goldspink *et al.* 1991). If this inactive muscle is simultaneously subjected to continuous electrical stimulation at 10 Hz, the imposed activity fails to prevent the atrophic changes from occurring. Hence, the length at which a muscle operates is also important in relation to protein turnover and muscle size.

Increased activity

Endurance exercise. With long-distance running, swimming or bicycling, the primary adaptation in skeletal muscle appears to be an enhancement of fatigue resistance. This is achieved by increasing (i) the capillary density to reduce diffusion distances, (ii) the number of mitochondria to generate ATP by the more efficient process of oxidative phosphorylation, and (iii) the economical utilisation of ATP (Saltin and Gollnick, 1983). Hence, significant alterations in the metabolic status of the muscle occur, without dramatically changing its overall size and power output. As already indicated, some hypertrophy may occur in the slow

res, but this is often offset by atrophy in some of the fast fibres (Goldspink, 1980).

In man, the effects of exercise on the size of the heart are usually the reverse of those on the skeletal musculature. That is, it is endurance rather than resistance exercise that produces cardiac hypertrophy (Urhausen and Kindermann, 1989). These observations have usually, but not always consistently, been confirmed in animal exercise models. Also in contrast to the effects of endurance activity on skeletal muscle, there appears to be little or no dramatic change in the mitochondrial density in cardiac muscle (Gollnick and Ianuzzo, 1972).

Alterations in mitochondrial density. Over the past two decades numerous workers have reported increases in both the number of mitochondria and the specific activities of mitochondrial enzymes in skeletal muscles of both animals and humans in response to endurance exercise (Saltin and Gollnick, 1983; Booth and Thomason, 1991). For example, in skeletal muscles of animals the mitochondrial enzyme activities associated with fatty acid oxidation, the respiratory chain and citrate synthetase have been reported to increase by 100%, while Krebs tricarboxylic acid cycle enzymes increase by 50%, in response to 2 h of treadmill running per day (Booth and Thomason, 1991). Perhaps not surprisingly, such adaptive responses vary in relation to the duration, frequency and intensity of the exercise undertaken. That is, the magnitude of the changes in mitochondrial enzymes increases roughly in proportion to the time spent running. Citrate synthetase activity increased by 15, 57 and 128% above control values with running times of 10, 30 and 120 min, respectively. Similarly, for the same running times muscle cytochrome *c* activity increased by 12, 30 and 90%, respectively, above control values (Fitts *et al.* 1975). However, running times beyond 120 min failed to produce any further increments. Nonetheless, the same increases in cytochrome *c* concentrations could be achieved by using faster running speeds coupled with shorter running times. Presumably this results in the recruitment of more motor units. Hence, mitochondrial density appears to be sensitive to both the duration and the intensity of exercise.

These increases in mitochondrial protein represent only a small proportion of the intracellular protein mass. Nonetheless, it is an extremely important adaptive change that is maintained as long as the training stimulus persists. Should the exercise be terminated, however, a rapid return to the non-trained state ensues. Likewise, inactivity arising from denervation or immobilisation causes precipitous decreases in the muscle's oxidative capacity. For obvious reasons, these effects are more pronounced in slow fibres than in fast ones.

Over the past 20 years, chronic electrical stimulation has been extensively used to good effect in completely transforming fast skeletal muscles into slow ones. Innumerable induced changes in the morphological, metabolic and contractile characteristics have been documented (see reviews by Salmons and Henriksson, 1981; Pette and Vbrova, 1985). When viewed in the context of exercise, chronic electrical stimulation must be regarded as a gross exaggeration of endurance tivities. Chronic electrical stimulation, unlike running, simultaneously recruits

Table 3. *Endocrine-induced changes in protein turnover in skeletal and cardiac muscle*

Hormone	Protein synthesis	Protein breakdown	References
Anabolic			
Insulin	+	−	1–4
IGF-1 and IGF-2	+	−	3
Thyroid	+	+	2–4
Growth hormone	+	0	3
Catabolic			
Glucocorticoids	−	+ or −	1, 3, 4
Glucagon	−	0	1, 4
Unresolved			
Catecholamines	Unclear		4

Insulin-like growth factors (IGF-1 and IGF-2).

Increases (+), decreases (−) or no changes (0) in the hormonally induced changes in the rates of protein synthesis and protein breakdown.

References: 1, McNurlan and Garlick (1989); 2, Barrett and Gelfand (1989); 3, Kettelhut *et al.* (1988); 4, Sugden and Fuller (1991).

all of the motor units in a muscle: this continues for 12–24 h a day, unlike running, which lasts for approximately 2 h per day (Booth and Thomason, 1991). Such differences are reflected in the type and rapidity of the changes in the muscles. For example, a greater fibre type transformation from fast to slow occurs with the electrical stimulation regime. However, this transformation is accompanied by a substantial loss of muscle mass and an eightfold drop in power output (Salmons and Jarvis, 1990), changes never seen with endurance exercise. Although changes in mitochondrial density are found in response to both chronic electrical stimulation and endurance exercise, the changes induced by electrical stimulation are enormous compared with those induced by endurance exercise. For example, continuous (24 h per day) electrical stimulation of fast muscles for 21 days increases citrate synthetase and cytochrome oxidase mRNA levels and activities by 220–650 %. By comparison, changes in the same enzymes in muscles after 2 weeks of running for 1.7 h per day were 25–60 %, i.e. approximately one-tenth of the changes induced by chronic electrical stimulation (Booth and Thomason, 1991).

The precise mechanisms responsible for the important increases in oxidative capacity in response to sustained activity remain unclear. Several hormones are known to modify the rates of protein turnover in skeletal and cardiac muscle (Table 3), and the circulating concentrations of many of these hormones are known to be either increased (e.g. glucagon, cortisol and catecholamines) or decreased (e.g. insulin) by exercise. Whilst a combination of haemodynamic and endocrine changes may possibly be important in relation to the enlargement of th

part, the induction of the increased oxidative capacity of skeletal muscle almost certainly is not. The effect can clearly be localised to individual muscles, which strongly suggests the involvement of myogenic, rather than general systemic, factors. This hypothesis is supported by the following observations. If humans are subjected to a one-legged form of exercise, the number of mitochondria in the muscles of the exercised limb increases, but that in the non-exercised contralateral leg does not (Saltin and Gollnick, 1983). Canoeists also possess higher specific activities of mitochondrial enzymes in the muscles of their arms, as compared with their legs. The reverse is true of cyclists. These observations are supported by animal experiments, with similar adaptive changes in oxidative capacity found in muscles of normal, hypophysectomized, thyroidectomised and diabetic animals in response to endurance exercise (Gollnick and Ianuzzo, 1972).

Precisely what factor(s) in the contracting muscle induces the increases in mitochondrial density is unclear. The pattern of usage, hypoxia, low pH, increased cyclic AMP concentration *via* the induction of β-receptors, the general flux of substrates through the mitochondria and/or the concentrations of ATP and/or creatine phosphate have all been suggested (Saltin and Gollnick, 1983; Booth and Thomason, 1991).

Despite these well-established adaptive changes in the mitochondrial enzymes, surprisingly little information is available about the events occurring at the level of transcription and translation. Results from experiments using chronic electrical stimulation for 21 days indicate that increases in both the mRNA concentration (110%) and the activity (310%) of the VIC subunit of cytochrome oxidase were induced (Williams *et al.* 1987). Similar increases in the mRNA and enzyme activity levels have been found for citrate synthetase. However, in the study of Williams *et al.* (1987) the increased concentration of mRNA encoding for the enzyme lagged behind the observed increase in its specific activity (Seedorf *et al.* 1986). Hence, although the rate of transcription was clearly enhanced in both studies, the increases in the enzyme activities were either greater than, or preceded, the amplifications of the mRNA. It has therefore been concluded that increases in the rate of synthesis per ribosome and/or decreases in the rates of enzyme degradation were induced. Although possible, such interpretations based on these data should be made with some caution, since the quantification of mRNA bands from Northern blot analysis is considerably less precise than in most enzyme assays. Hence, quantitative comparisons of this nature may not be very accurate or meaningful.

Induced changes in protein turnover. Although a few investigations indicate that protein turnover in striated muscles is altered by exercise, these represent an exceedingly small proportion of the vast literature concerned with exercise. Within this small group of studies, most investigators have concentrated on endurance exercise. At the outset it should be pointed out that differences in methodology and the precise timings when measurements were made, e.g. during or after the bouts of exercise, make it desirable to use some caution when summarising the available data.

A variety of studies has been performed on man and animals. Collectively, these suggest that there is a negative nitrogen balance in skeletal muscles during endurance exercise, which is restored to a positive balance during the recovery phases. Hence, it is possible that any major quantitative or qualitative adaptations in muscle proteins occur after, rather than during, the bouts of exercise.

Changes in protein synthesis. In man, whole-body protein synthesis (*in vivo*) has been calculated from the flux and oxidation of amino acids (e.g. radioactive or stable isotopes of leucine or glycine) administered either orally or by constant intravenous infusion. Good agreement can be found across several studies, strongly suggesting that whole-body protein synthesis is decreased during acute exercise (Haralambie and Berg, 1976; Rennie *et al.* 1981; Wolfe *et al.* 1982). Rennie *et al.* (1981) also observed that when rates of protein synthesis were measured after completion of the exercise (i.e. during the recovery phase) greater values than those measured during, or before, exercise were recorded.

Confirmatory evidence, indicating that muscle protein synthesis also decreases during exercise, comes mainly from animal experiments. Here, mixed, soluble, total myofibrillar and actin proteins were found to be less rapidly synthesised in the skeletal muscles of rats immediately following treadmill running (Bates *et al.* 1980) or swimming (Dohm *et al.* 1980, 1982*a*). Generally, the longer and more intense the bout of exercise, the greater the suppression of the synthesis rates (Dohm *et al.* 1982*a*).

In animals where the stress on the left ventricular wall is elevated as a consequence of pressure overloading, the number of ribosomes and the rate of protein synthesis increase in association with the enlargement of the heart (Watson *et al.* 1989; Xenophontos *et al.* 1989). These changes probably occur through cyclic-AMP-dependent protein kinase mechanisms (Xenophontos *et al.* 1989) and are accompanied by a shift in the myosin isoforms from the adult V_1 towards the embryonic V_3 form (see review by Swynghedauw, 1986). The shift in the cardiac myosin isoforms in this experimental model is, however, the reverse of that expressed in response to endurance training. Whether the rats are made to swim or run, the myosin shift is in favour of the V_1 form, thereby increasing the V_1:V_3 ratio still further (Pagani and Solaro, 1983). Although the adaptive hypertrophy of the heart does not appear to be dependent on the presence of thyroxine or growth hormone, hypothyroid animals do not show the shift towards the V_1 form of myosin upon swimming. Hence a combination of haemodynamic and endocrine (Table 3) changes may be responsible for the increase in cardiac protein synthesis and concomitant hypertrophy of the heart.

Changes in protein degradation. Here the changes in response to endurance exercise are much less clear cut. Whole-body protein degradation, as measured by urinary nitrogen excretion, is generally increased. This is equally true of athletes, after either short strenuous (Decombaz *et al.* 1979) or lengthy bouts of running (Refsum and Stromme, 1974; Refsum *et al.* 1979; Haralambie and Berg, 1976), and non-athletes, after 3–4 h on a treadmill (Rennie *et al.* 1981; Dohm *et al.* 1982*b*). At first sight it might seem reasonable to assume that whole-body rates

[redacted]ch as these mainly reflect the changes within the musculature, particularly since it represents 50–70 % of the body's protein mass (Goldspink *et al.* 1985). Not all of the data, however, support this assumption. For example, decreased rates of urinary excretion of 3-methylhistidine were found in the same subjects who exhibited increased whole-body rates during exercise (Rennie *et al.* 1981). Radha and Bessman (1983) also reported lower rates of 3-methylhistidine excretion with exercise. Hence, while these authors are describing an increased rate of protein degradation in the whole body, the implication is that the catabolism of myofibrillar proteins in muscle is suppressed. The situation is even further confused with Decombaz *et al.* (1979), Plante and Houston (1984*a*,*b*) and Refsum *et al.* (1979) finding no change, and Dohm *et al.* (1982*b*) reporting an increased urinary output of 3-methylhistidine.

This situation illustrates a major methodological problem. Whilst there are reliable methods available for measuring protein synthesis *in vivo* (McNurlan *et al.* 1979), no comparable techniques are yet available to allow a meaningful measurement of protein degradation in muscle or other body tissues *in vivo* (see above). 3-Methylhistidine has the advantage of being a non-reutilisable amino acid when released from actin and myosin. However, to use its rate of excretion in the urine as an index of muscle actomyosin breakdown is fraught with potential problems (Rennie and Millward, 1983). For example, a disproportionate amount of this amino acid comes from a small, but rapidly turned over, pool of 3-methylhistidine in smooth muscles of the body. Even if such problems did not exist, at best the excretion of 3-methylhistidine would reflect changes in the whole skeletal musculature and not individual, or small groups of, muscles that might be particularly relevant to some forms of exercise. Hence, the application of this technique is limited.

Unfortunately, animal experiments, which clearly do provide direct analysis of individual muscles, have not always succeeded in clarifying this situation. One hour after swimming, the amount of tyrosine released from muscles in rat perfused hindquarter preparations increased, indicating accelerated protein degradation (Dohm *et al.* 1980). This conclusion was further supported by increased rates of 3-methylhistidine excretion in the urine (Dohm *et al.* 1982*b*).

An alternative approach involves measuring changes in the activities of various proteolytic enzymes. Several of these have been measured in a number of skeletal muscles following exercise. Clearly, it is unlikely that changes in the activity of any one enzyme will accurately reflect the changes in the degradation of mixed muscle proteins. However, as mentioned above, the activities of some acid hydrolases, e.g. cathepsins B and D, do appear to parallel changes in the average rates of degradation, as measured directly *in vitro* or calculated from the measured rates of synthesis (*in vivo*) and muscle growth (Goldspink *et al.* 1983; Goldspink and Lewis, 1987).

In one study Dohm *et al.* (1980) found the activity of cathepsin D, but not of Ca^{2+}-activated or alkaline proteinases, to be increased in muscles of the rat [redacted]llowing running to exhaustion. Similarly, in several studies undertaken by a

Swedish group (Salminen and Vihko, 1980, 1981, 1982; Salminen *et al.* 198[illegible] increased activities of cathepsin D and dipeptidyl aminopeptidase were measured in hindlimb muscles of rodents after treadmill running. In contrast, in the same muscles small decreases in the activities of alkaline and myofibrillar proteolytic enzymes were found. Severe exercise, as used in some of these studies, can result in muscle damage and inflammation. Such injuries are known to attract phagocytic cells which, being rich in lysosomes, may conceivably account for some, if not all, of the increased acid hydrolase activities.

No changes were observed in the activities of any of these proteolytic enzymes when measured in the heart after endurance exercise (Salminen and Vihko 1980, 1981; Kainulainen *et al.* 1987).

The weight of the evidence currently available suggests that protein synthesis in skeletal, but not cardiac, muscle is suppressed during endurance exercise. Moderate, but less well-established, changes in protein degradation may also contribute to the acute negative nitrogen balance experienced by the skeletal musculature during this sustained type of exercise.

Dynamic or high-resistance exercise

This type of exercise results in an increase in muscle bulk in excess of that occurring during normal developmental growth. A well-developed musculature is conspicuous both in individuals whose daily occupation involves heavy physical labour and in athletes whose competitive events involve dynamic work, e.g. weightlifting and field events. Strength training also appears to be effective in counteracting some of the morphological and functional changes in ageing human muscle (Klitgaard *et al.* 1990).

Resistance training in humans usually consists of intermittent sessions of low-frequency repetitions using heavy loads, e.g. 66–75 % of maximal voluntary contraction. Lengthy periods of rest are usually interspersed between the training sessions. Generally, such exercises lead to a relatively slow enlargement (approximately 0.1–0.2 % per day) of the muscles in question; the major emphasis being on increasing force output rather than producing changes in fatigue resistance. So, in contrast to endurance training, resistance exercise is often accompanied by decreases in mitochondrial volume and mitochondrial enzyme activities. In some instances, particularly in non-trained individuals, the increased workloads can enhance force development without altering the cross-sectional area of the muscle. This phenomenon may arise from functional adaptations within the central nervous system, e.g. increasing the capacity to activate and recruit motor units, rather than inducing changes within the muscle itself (Milner-Brown *et al.* 1973). Generally, high-resistance exercises enhance muscle strength through increases in cross-sectional area. As a consequence, the capacity to develop tension per unit cross-sectional area is unaltered by such training regimes (Saltin and Gollnick, 1983), implying that, at the molecular level, the tension developed per crossbridge remains constant. Also, as the fibre number is fixed soon after birth, and[illegible]

dependent of activity levels, any increases in muscle strength must be achieved through the hypertrophy of existing fibres (Goldspink, 1980).

Changes in protein turnover. The primary long-term response to resistance exercise is an increase in the myofibrillar protein content and, hence, muscle mass. Even fewer studies than for endurance exercise have been undertaken to determine the nature of the changes in protein turnover that explain the increased muscle mass.

In man, the recent work of Tarnopolsky *et al.* (1988) suggests that during habitual training body builders and endurance athletes require 1.1 or 1.7 times more protein, respectively, than non-athletes. It has been suggested that the greater dietary protein requirement of the endurance athlete is to compensate for the net whole-body protein catabolism that occurs during exercise (see above). No such increase in protein degradation occurs in elite body builders, whose urinary excretion of urea or 3-methylhistidine was not affected by their training (Hickson *et al.* 1986).

To my knowledge only one study has attempted to examine the changes in protein synthesis rates in man while undergoing resistance exercise (Tarnopolsky *et al.* 1991). By infusing the stable isotope L-[1-^{13}C]leucine, measurements of whole-body protein synthesis were made during, and 2 h after, a bout of circuit-set exercises. Each set of exercises, consisting of a series of bench, leg, triceps and military presses, sit-ups, latissimus pull-downs and biceps curls, was performed for 30 s with 2 min rest in between, for a total of 1 h. No changes in whole-body protein synthesis were noted either during, or after, these resistance exercises. The fact that whole-body, and not muscle, rates of protein synthesis were measured once again reflects the difficulties associated with studying humans, as compared with animals.

Animal models mimicking this type of high-resistance exercise are rare and sometimes of questionable value when compared with the physical activities undertaken by humans (Booth and Thomason, 1991). Nonetheless, some models do come close to mimicking some human activities, e.g. weightlifting. Rodents and cats have been trained to pull down weighted food baskets, or to jump onto elevated food containers, in order to feed. During 2 weeks of such a jumping/feeding regime, both fast (i.e. extensor digitorum longus, EDL) and slow (i.e. soleus) muscles increase (40–70 %) their rates of growth, compared with muscles of non-jumping animals (Watt *et al.* 1982). This greater accumulation of protein correlated with 15–40 % increases in the diameters of all three constituent fibre types (SO, FOG and FG), without altering the total number of fibres present (Watt *et al.* 1982). The same regime was later shown to delay the developmental conversion of FOG into SO fibres, thereby influencing maturational changes within the soleus muscle of juvenile rats (Watt *et al.* 1984). Despite the similarity of the responses of these two hindlimb muscles to this jumping exercise, the accelerated growth arose in different ways. The greater accumulation of protein in the EDL muscle resulted from a 28 % increase in protein synthesis with no change in protein degradation. The reverse was true of the soleus; the accelerated growth correlated with a 38 %

decrease in protein degradation, but no change in protein synthesis (Watt *et al.* 1982). The extensor carpi radialis is an intermediate type of muscle located in the forelimb. Its hypertrophy presumably arose from increased activity in gripping and hanging onto the food container whilst feeding. The additional growth in this mixed muscle resulted from a complementary increase (12 %) in protein synthesis and a decrease (11 %) in protein breakdown, i.e. a response intermediate between those of the two extreme muscle types in the hindlimb (Watt *et al.* 1982).

In another animal model, a single bout of either concentric or eccentric contractions was imposed on the rat tibialis anterior muscle. Although the concentric contractions induced a smaller degree of hypertrophy than the eccentric training, in both situations the synthesis of mixed and myofibrillar proteins increased by 50–60 %, 12–48 h after exercise (Wong and Booth, 1990*a*,*b*).

The rapid and dramatic growth of the overloaded soleus (Goldspink *et al.* 1983) and plantaris (Ianuzzo and Chen, 1979) muscles in response to tenotomy of the gastrocnemius muscle can also be viewed as an example of resistance work. With the functional loss of this powerful synergist muscle, the soleus and plantaris muscles are initially stretched, with the ankle being restrained for far longer than normal in plantar flexion. This particular experimental model does, of course, differ from the weightlifting regimes in that it is more extreme, with a continuous and not intermittent form of muscle loading. The resultant muscle hypertrophy under these conditions arises from antagonistic increases in the average rates of both protein synthesis and protein degradation; the former rate increased more rapidly and to a greater extent than the latter (Goldspink *et al.* 1983). The finding of increased degradative rates has been further supported by observed increases in the specific activities of cathepsins B (100 %) and D (40 %) in the soleus muscle 3 days after tenotomizing the gastrocnemius (Goldspink and Lewis, 1987).

One other form of resistance exercise concerns fast twitch muscles simultaneously stretched and electrically stimulated at 10 Hz. Although extreme, this type of isometric work is worthy of brief mention. On the one hand, it produces a massive (27–40 %) and extremely rapid growth of the muscle through large increases in the rates of protein synthesis (Table 4). On the other hand, these combined mechanical stimuli cause changes in gene expression that are reflected in fast to slow fibre type conversions, with presumed concomitant slowing of contractile characteristics (Williams *et al.* 1986). By contrast, when the same two muscles were simply electrically stimulated at the same low frequency and for the same period (i.e. 3 days), no significant changes in either muscle size or protein synthesis were observed (Table 4). However, in both experimental conditions the rate of proteolysis, as determined by the activities of cathepsin B and dipeptidyl aminopeptidase, increased (Table 4), suggesting increased protein catabolism. The changes in protein degradation are, however, more than offset by the elevated rates of synthesis in the stretched/stimulated muscles; the net effect is rapid growth. By contrast, since degradation is initially increased whilst synthesis remains unchanged (Table 4) in those muscles that were only electrically stimu-

Table 4. *Acute changes in muscle size and protein turnover in response to 3 days of mechanical stimulation*

	Percentage changes relative to control muscle				
				Proteolysis	
Muscle	Wet mass	Protein mass	Protein synthesis	Cathepsin B	Dipeptidyl aminopeptidase I
EDL					
Stretched and stimulated	+40	+28	+284	+307	+243
Stimulated only	−4	−6	+4	+60	+49
AT					
Stretched and stimulated	+30	+27	+126	+106	+90
Stimulated only	+2	−17	−4	+59	+18

These results are unpublished observations from S. K. Winterburn, J. Easton, D. Mantle and D. F. Goldspink.

Rabbit extensor digitorum longus (EDL) and anterior tibialis (AT) muscles were either electrically stimulated at 10 Hz, or simultaneously stretched and stimulated at the same frequency, for 3 days. All measurements relating to growth and protein turnover were made as previously reported (Lewis *et al.* 1984; Goldspink and Lewis, 1987).

These values are presented as percentage changes, as compared with the measurements (N=5) made on internal control muscles of the contralateral limb.

lated, in the longer term muscle atrophy would be predicted. This would appear to be the case after a few weeks (Salmons and Jarvis, 1990).

Although not commanding total agreement, most studies suggest that the muscle hypertrophy arising from resistance exercises involves increased rates of protein synthesis, at least after, if not during, the bout of exercise. This change may be coupled with either antagonistic (i.e. increased) or complementary (i.e. decreased) alterations in the rates of protein degradation. Further studies are clearly required to clarify this unsatisfactory situation.

Conclusions

Despite the vast literature on exercise, plenty of scope still exists for investigating further the effects of the different types of exercise on protein turnover in skeletal and cardiac muscle. Such studies should help to clarify some of the conflicting and unresolved issues, e.g. the precise changes induced in muscle protein degradation. Further, the question of what changes occur in the synthesis and turnover of individual muscle proteins has barely been embarked upon, let alone explained in terms of the events occurring at the level of transcription and translation. Similarly, the underlying systemic (e.g. endocrine and haemodyna-

mic) and/or myogenic (e.g. mechanical stimuli, such as stretch, and patterns of usage) mechanisms still need to be unravelled.

Although the adaptations to exercise are clearly relevant to animals and their ability to survive, more care should be given to the design of those animal experiments intended to mimic the activities of man (Booth and Thomason, 1991). Even more meaningful would be to study man himself, including his tissue responses to exercise, more intensively than hitherto. The development of better techniques, e.g. the flooding dose and/or shorter constant infusion methods using stable isotopes (McNurlan and Garlick, 1989) coupled with tissue biopsies, should enable accurate measurements of rates of protein synthesis *in vivo* to become more commonplace. In contrast, the inability to measure protein degradation *in vivo* with any real confidence remains a major obstacle in advancing studies in both man and animals. Discernible changes in whole-body rates of protein degradation in man, as well as in animals, clearly indicate the importance of protein catabolism in regulating the size of tissues and the whole body (McNurlan and Garlick, 1989).

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