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REVIEW

How nutrition and exercise maintain the human musculoskeletal mass

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Abstract

In this article we review some of our recent work concerning the effects of nutrition and exercise on protein synthesis and signal transduction in human musculoskeletal tissues. A great deal of new information is being generated by the application of recently refined techniques for measuring protein turnover. The field remains one that is largely descriptive but increasingly we are beginning to discern mechanisms underlying lean tissue maintenance, growth and wasting especially as multidisciplinary tools are applied to its study. Several types of exercise and nutrition are potent stimuli for protein synthesis in skeletal muscle. By contrast, collagen in the extracellular matrix in muscle and tendon appears to be mechanically but not nutritionally sensitive. The rates of collagen turnover in a variety of tissues are sufficiently high to account for a sizeable proportion of whole body protein turnover. One of the most recent surprises is the high turnover rate of human bone collagen and its anabolic response to feeding. As our understanding of the normal physiology of these processes advances, we become better able to construct testable hypotheses concerning the effects of ageing and disease on the musculoskeletal mass. Current evidence suggests that one of the major problems with loss of muscle during ageing is an inability of the tissue to respond adequately to increased availability of nutrients.

Key words bone; collagen; protein synthesis; sarcopenia; signal transduction; skeletal muscle.

Introduction

In healthy adult men and women the musculoskeletal system makes up about half of the total body weight (though it is slightly less in women). Much of the variation in the size of the muscle mass is explained by inheritance: heritability estimates for lean body mass (which is linked to muscle mass) range from 0.52 to 0.77 (Arden & Spector, 1997; Hanisch et al. 2004; Schousboe et al. 2004). The muscle mass of an individual depends on the number and size of muscle fibres. It is likely that, in particular, the number of fibres is inherited. Human muscle fibre numbers vary greatly between individuals [between 3 and 9×10^5 fibres were counted in the vastus lateralis (Lexell et al. 1988)] but change little or not in response to training or other environmental stimuli (Matoba & Gollnick, 1984; Abernethy et al. 1994). This suggests that the variation in fibre number between human beings is mainly due to inheritance. Fibre number can, however, change significantly in animal models, where larger gains in muscle size have been achieved with radical interventions such as synergist ablation (Kelley, 1996). We have started to identify the mechanisms that determine the inheritance of muscle mass and fibre numbers. The most prominent example is the myostatin gene. Transgenic mice lacking functional myostatin display a large muscle mass (McPherron et al. 1997) and 'natural' knockout mutations in the myostatin gene greatly increase muscle mass in cattle (McPherron & Lee, 1997) and human beings (Schuelke et al. 2004).

The aforementioned high heritability estimate for lean body mass masks the fact that resistance training can greatly increase human muscle mass, especially when

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combined with nutritional or pharmacological interventions. Resistance training stimulates muscle protein synthesis (MPS) and promotes fibre hypertrophy as will be discussed later in this review. To conclude, heritability does probably determine the size potential of a muscle by setting fibre numbers. Environmental stimuli mainly regulate the size of muscle fibres.

What is the link between muscle size and protein metabolism? Muscle size, protein synthesis and breakdown are closely linked because protein is the major component of muscle. The most abundant muscle proteins are myosin, actin and collagen, which together account for about 85% of the protein-bound amino acid. Protein metabolism can be investigated using isotopically labelled tracers, a technique pioneered by Rudolph Schoenheimer in the USA in the 1930s. As a result of Schoenheimer's work the concept of a division between exogenous, dietary and endogenous tissue metabolism was replaced with that of the 'dynamic state of metabolism'. According to this concept, amino acids in the metabolic pool are continually taken up by tissues for protein synthesis and released because of protein breakdown. The use of stable (i.e. non-radioactive) isotopes allowed researchers to use labelled tracers safely to investigate human protein turnover (Rennie, 1999). The ability to measure protein synthesis in human being(s) is important because human muscle protein turnover is considerably slower than in rodents and is not insulin-sensitive to the same degree. The fractional synthesis rate for mixed muscle in 4-day starved rats is ~4% per day (Bates et al. 1983) but only ~1% per day in starved human muscle (Cuthbertson et al. 2005).

Being able to use tracer studies to measure alterations in protein turnover in human tissues enables us to provide explanations for the changes in tissue mass observed over longer periods. Knowledge of protein synthesis and breakdown rates immediately raises new questions concerning the control of synthesis and breakdown themselves. Such questions can be addressed by measuring the activation of signal transduction pathways. Better techniques for obtaining samples or measuring aterio-venous differences across tissues and more sensitive and precise mass spectrometric assays have enabled many advances to be made, particularly in the last 20 years. Questions we wish to explore in this review include:

1 What are the effects of feeding and exercise on muscle, tendon and bone protein turnover?

2 What mechanisms mediate the response?

3 What causes sarcopenia, the normal loss of muscle mass and strength during ageing?

Muscle and feeding

The following sections focus primarily on the contribution of the Rennie group to this area of research. A key, early observation was that MPS can be doubled by feeding mixed nutrients (Rennie et al. 1982). We subsequently showed that administration of amino acids alone, and in circumstances which caused no increases in blood glucose or insulin availability, could mimic a large part of the effect of mixed food on human MPS (Bennet et al. 1989). Data from a recent study suggest that MPS is mainly linked to extracellular rather than intracellular amino acid availability (Bohe et al. 2003); we also showed that the stimulation of MPS does not require insulin (Bohe et al. 2003; Cuthbertson et al. 2005; Greenhaff et al. 2005). The period of MPS stimulation by amino acids must be time limited because nutritional interventions could otherwise be used to promote muscle hypertrophy as effectively as resistance training. We found that doubling the availability of amino acids increases MPS after a latent period of about 30 min by about 3.5 times the fasted rate, with larger increases for myofibrillar than for sarcoplasmic protein. Within 1 h of continued amino acid availability, the rate of MPS starts to decline, approaching baseline values after about 2.5–3 h of amino acid administration (Bohe et al. 2001). The mechanism responsible for the reduction of MPS despite the ongoing presence of amino acids is currently unknown. Excess amino acids are diverted to metabolism in the liver as substrates for ureagenesis and gluconeogenesis. It seems likely to us that the normal temporal sequence of feeding and fasting throughout the diurnal cycle enables MPS during relatively short, fed periods to replace protein broken down during the periods between meals and hence accomplish the regular renewal of structural and functional components of the muscle mass at least.

Muscle and exercise

Exercise has profound effects on muscle protein turnover during and after exercise. Acute muscle contraction decreases MPS in some rodent (Bylund-Fellenius et al. 1984) and human muscles (Rennie et al. 1981). After exercise, MPS rebounds, especially if foods containing protein or amino acids are administered (Chesley et al.

1992; Biolo et al. 1997). Resistance or intense dynamic exercise increases MPS for much longer than the 2.5–3 h that can be achieved with feeding (Bohe et al. 2001). For example, MPS was still elevated 72 h after 1 h of one-legged kicking exercise at 67% of the maximum workload (Miller et al. 2005). Protein breakdown also increases post exercise (Phillips et al. 1997) and it is likely that both intense exercise and feeding are required for protein accretion (Tipton et al. 1999). The timing of the feeding is important because consuming protein directly after exercise is likely to be more effective for promoting muscle growth than ingesting the same meal hours later (Esmarck et al. 2001; Levenhagen et al. 2001). Repeated high-resistance exercise will result in a measurable increase of muscle size and strength. DeLorme was the first to distinguish in a scientific paper between the adaptations to resistance and endurance exercise; he advocated the use of high-resistance exercise rather than endurance exercise for stimulating muscle hypertrophy (DeLorme, 1945). The American College of Sports Medicine (ACSM) currently recommends sets of resistance exercise with an 8–12 repetition maximum for promoting muscle growth and strength in novices (Kraemer et al. 2002).

Hormonal influences on muscle

Exercise increases muscle growth mainly via local mechanisms: training a muscle will stimulate growth in that muscle but will have no or only small effects on the growth of other muscles. This suggests that systemic factors are not essential for the muscle growth response to exercise. Some hormones additionally increase MPS and the two most obvious candidates as humoral modulators of the muscle mass are growth hormone and testosterone. Administration of growth hormone to children markedly increases the accretion of lean body mass (Gregory et al. 1991), presumably by inducing IGF-1 production in liver and muscle (Butler & Le Roith, 2001). However, in adults growth hormone appears to have no stimulatory effect on MPS (Rennie, 2003), although the clinical condition of patients with acromegaly suggests it may have marked stimulatory effects, even in adulthood, on connective tissue growth presumably including collagen in muscle, tendon and bone. Testosterone has a dramatic effect on the size of skeletal muscle fibres and on gross muscle bulk (Forbes, 1985) and anabolic steroids have been misused by athletes for this effect.

Signal transduction mechanisms that mediate the response of MPS to nutrients and exercise

Protein synthesis and growth are regulated by signal transduction proteins whose homologues can often be found in yeast, fly, fish, rodents and human being(s), suggesting a high degree of evolutionary conservation. The major mechanism of signal transduction is the phosphorylation or dephosphorylation of proteins. It is currently estimated that about one-third of all proteins contain covalently bound phosphate (Cohen, 2002) and genes for 518 protein kinases have been identified in the human genome (Manning et al. 2002). The number of protein phosphatases probably also lies in their hundreds. These facts demonstrate the complexity of the signal transduction network that governs the fate of cells in the human body.

The synthesis of a protein depends on the transcription of DNA into mRNA and on the translation of that mRNA into protein. Nutrition, exercise and hormones affect both transcription and translation in muscle. DNA microarray experiments show that insulin (Rome et al. 2003), endurance (Mahoney et al. 2005) and resistance exercise (Zambon et al. 2003) change the concentrations of hundreds of different mRNAs in human muscle within hours. It is impossible to review the mechanisms of transcriptional regulation here because the transcription of the ~25 000 genes within the human genome depends on a plethora of regulatory mechanisms. The second step is translation, which is the actual protein synthesis. It involves (1) translation initiation, (2) elongation of the peptide chain and (3) termination. During initiation the ribosome complex is assembled from 60S and 40S ribosome subunits, about 80 ribosomal proteins, mRNA, initiator tRNA and eukaryotic initiation factors (eIFs). Elongation involves the synthesis of peptide bonds between amino acids; it is controlled by eukaryotic elongation factors (eEFs). Translation of the mRNA into peptide is terminated once the stop codon of the mRNA has been reached by the ribosome.

A positive regulator of translation and muscle size is IGF-1. IGF-1 was discovered as a growth factor that mediated the effect of growth hormone. Systemic IGF-1 is secreted by liver into the bloodstream and acts as a growth-stimulating second messenger for growth hormone (Butler & Le Roith, 2001). Subsequently, it was shown that specific IGF-1 splice variants were produced by muscle. A stretch-responsive splice variant named mechano-growth factor (MGF) was discovered

in rabbit muscle (Yang et al. 1996). The response of IGF-1 splice variants to resistance exercise in human muscle is currently unclear. Resistance exercise increases MGF but not IGF-1Ea mRNA significantly 2.5 h after exercise in young but not old muscle (Hameed et al. 2003). Others have found that the mRNA of IGF-1 splice variants remains unchanged or decreases after resistance exercise (Psilander et al. 2003).

IGF-1 has been shown to activate translation and MPS via the protein kinase B (PKB)-tuberin(TSC2)-mammalian target of rapamycin (mTOR) signal transduction cascade (Bodine et al. 2001; Rommel et al. 2001). We are not able to review the whole cascade here but will focus on major signalling proteins in this pathway. Increasing the activity of the IGF-1 target PKB in a muscle by transgenic methods causes hypertrophy in rodent muscle (Pallafacchina et al. 2002; Lai et al. 2004). PKB works by phosphorylating TSC2 at phopshorylation sites (Inoki et al. 2002) that are distinct from the AMP-activated kinase (AMPK) phosphorylation sites (Inoki et al. 2003). PKB phosphorylation of TSC2 will lead to the activation of mTOR and increase protein synthesis. By contrast, phosphorylation of TSC2 by AMPK as a result of high [AMP] (because of exercise, hypoxia or nutrient depletion) will inhibit mTOR and protein synthesis (Inoki et al. 2003). This might explain why IGF-1 stimulates protein synthesis whereas acute exercise (which activates AMPK) inhibits protein synthesis (Rennie et al. 1981; Bylund-Fellenius et al. 1984). Also, the selective activation of AMPK by endurance training-like interventions and activation of PKB-TSC2-mTOR signalling by resistance training-like interventions might explain the specific adaptations to these types of exercise in rodent muscle (Atherton et al. 2005). More studies are needed to test whether this hypothesis applies to human muscle.

mTOR, its downstream targets and translation are all also activated by amino acids via mechanisms that do not involve PKB (Proud, 2002). The amino acid sensing mechanism is currently unknown but progress is being made towards its identification. It will be interesting to see whether the amino acid sensor is intracellular or extracellular as was suggested in a recent study involving human subjects (Bohe et al. 2003). mTOR regulates the rate of translation mainly via eIFs but also via eEFs and other signalling proteins. Here, we focus on p70 S6 kinase (p70 S6k), an important target of mTOR. The knockout of p70 S6k in muscle cells results in atrophy, confirming that p70 S6k is a regulator of muscle size (Ohanna et al. 2005). High-resistance exercise in rats increases the phosphorylation of p70 S6k for several hours after exercise and stimulates fast muscle hypertrophy when repeated (Baar & Esser, 1999). p70 S6k and protein synthesis are still activated 24 h after resistance exercise in rats (Hernandez et al. 2000). The long-term activation of p70 S6k correlates well with the long-term increase of MPS after intense exercise, which can last for up to 72 h (Miller et al. 2005). More research is needed to see whether p70 S6k activation is the cause of the long-term increase of MPS after intense exercise.

Myostatin is a muscle growth inhibitor and in many respects is a mirror image of IGF-1. Transgenic and natural knockout myostatin mutations result in a large muscle mass in, for example, mice, cattle and human being(s) (McPherron & Lee, 1997; McPherron et al. 1997; Schuelke et al. 2004). The human example, a young child homozygous for a loss-of-function myostatin mutation, had a quadriceps area of 6.72 cm^2, which was more than twice the mean of age- and sex-matched controls (Schuelke et al. 2004). Muscle and serum myostatin have been reported to be lower in HIV-infected men (Gonzalez-Cadavid et al. 1998) and increasing systemic myostatin has been shown to cause cachexia in mice (Zimmers et al. 2002). It is unclear whether myostatin is involved in the regulation of muscle growth in response to resistance exercise or other environmental stimuli. Some reports show a decrease of myostatin mRNA or protein after resistance exercise (Roth et al. 2003; Zambon et al. 2003; Walker et al. 2004) but one paper does not support the finding (e.g. Willoughby, 2004). The mechanisms by which myostatin regulates muscle mass are unclear. Myostatin binds to activin receptors (Lee & McPherron, 2001), which leads to the phosphorylation of Smad2/3 ['Smad' stands for '(similar to) mothers against decapentaplegic homolog'] proteins. One study suggests that myostatin inhibits protein synthesis in cultured muscle cells (Taylor et al. 2001) but it is unclear whether myostatin reduces protein synthesis in human beings *in vivo*. It is equally unknown whether the reduction of protein synthesis occurs as a result of transcriptional or translational inhibition. Other reports show that myostatin inhibits the proliferation and differentiation of cultured muscle cells *in vitro* (Thomas et al. 2000; Langley et al. 2002).

Collagen turnover in musculoskeletal tissues

We now switch from muscle protein to collagen. Much is known about the cell biology and molecular biology

of collagen as a result of studies in animals and in tissue culture. The physiology, pharmacology and pathophysiology of collagen in the adult human body is, however, much less understood. For example, until recently we had no idea about the effects of the two most potent influences on muscle (namely nutrition and physical activity) on collagen turnover in musculoskeletal tissues. As a result of the development of methods which have enabled us to measure collagen synthesis in a wide variety of human tissues (skin, muscle, tendon, ligament and bone; Babraj et al. 2005a,b), we have begun to fill in some of the gaps. Some of the findings are surprising. First, collagen turnover appears to be remarkably fast compared with historical expectations. Previously we tended to think of much of the collagen in the body as being fixed but, for example in muscle, the fractional synthetic rate of collagen (presumably produced by fibroblasts in the extracellular matrix to form the endomysium and perimysium) is about half as fast as myofibrillar protein turnover whereas in tendon and ligament the values are as fast as the synthetic rate of myofibrillar proteins. Collagen turnover is likely to be a major contributor to whole body protein turnover. However, unlike both the myofibrillar and sarcoplasmic classes of muscle proteins, muscle collagen is not at all responsive to feeding (either as mixed nutrients or as amino acids alone; Babraj et al. 2005a). Despite the lack of responsiveness of muscle collagen synthesis to feeding, it is remarkably responsive to physical activity. Collagen is made by fibroblasts, which presumably do not show the increases in muscle metabolism observed in working muscle. It is thus unlikely that these changes are the result of increased ATP turnover and are much more likely to be the result of increased tension transmitted to the extracellular matrix by contracting muscle. It is interesting in this regard that collagen synthesis is similar in muscle after eccentric and concentric exercise (Moore et al. 2005).

Bone collagen synthesis

Almost nothing is known about bone collagen turnover in humans. The use of indirect markers such as procollagen peptides (which are used as markers of collagen synthesis) and degradation products of collagen such as N- and C-terminal telopeptides and the pyridinolones is non-specific, temporarily insensitive and imprecise. We have recently developed methods for the direct measurement of collagen synthesis and applied them, with novel results, to the study of human bone. We have shown that human bone collagen synthesis is about as fast as mixed muscle protein turnover (Babraj et al. 2005b). Human bone collagen synthesis is remarkably nutritionally responsive, showing a near doubling as a result of feeding. This has important implications, not only because tissues which show high rates of protein turnover tend to be relatively plastic and adaptable but also because of the possibility of testing new therapeutic approaches to decreasing bone wasting. Bone collagen turnover is obviously differently regulated from that in muscle and tendon with respect to nutrition but it is tantalizing to conceive of it being as influenced by physical activity as muscle and tendon collagen. The answer to this awaits further research.

Alterations in MPS during sarcopenia

Muscle mass may be lost for several reasons, including malnutrition, inactivity (cast immobilization, paraplegia), conditions with altered cytokine signalling (HIV, cancer, burns) and sarcopenia. In this last section we focus on sarcopenia (Greek: *sarx* flesh and *penia* loss), which is a special case of muscle wasting. Sarcopenia denotes the normal loss of muscle mass and strength during ageing; the term 'sarcopenia' had been coined to achieve a greater recognition of this common syndrome (Rosenberg, 1997). Muscle is lost at 0.5–2% per year beyond the age of 50. The reduction results from a loss of mainly type II fibres (Lexell et al. 1988), which is most likely secondary to a loss of α motor neurons during ageing (Kawamura et al. 1977). The loss of fast fibres is the major cause of sarcopenia but a plethora of other factors have been suggested to contribute to the decline in muscle mass (Narici et al. 2004). Altered muscle protein metabolism is a second, major cause. Protein turnover in the basal post-absorptive state in healthy ageing muscle is probably very similar to the rates found in younger adults (Volpi et al. 1998). Earlier reports of a decreased rate of basal muscle protein synthesis at old age may reflect the study of old, more frail and unhealthy individuals (Yarasheski, 2003). The situation is different for nutrient-stimulated protein synthesis as was recently shown by two groups: in both papers it was demonstrated that the rate of amino acid-stimulated muscle protein synthesis increased to lower rates in old (~70 years old) than in young male subjects (Guillet et al. 2004; Cuthbertson et al. 2005). Both groups also reported defects in signal transduction. Guillet et al.

(2004) reported that the general phosphorylation of muscle growth regulator p70 S6k did not increase in the old group after insulin and amino acid stimulation. Cuthbertson et al. (2005) found that amino acids led to smaller relative increases in the phosphorylation of mTOR at Thr2448, p70 S6k at Thr289, and 4E-binding protein 1 (4E-BP1; a translational regulator) at Ser37/46 in the old men when compared with young. Taken together, these results suggest that nutrients activate protein synthesis less in old muscle owing to a defect that lies upstream of mTOR-p70 S6k/4E-BP1 signalling.

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