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Plasticity of human skeletal muscle: gene expression to *in vivo* function

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Human skeletal muscle is a highly heterogeneous tissue, able to adapt to the different challenges that may be placed upon it. When overloaded, a muscle adapts by increasing its size and strength through satellite-cell-mediated mechanisms, whereby protein synthesis is increased and new nuclei are added to maintain the myonuclear domain. This process is regulated by an array of mechanical, hormonal and nutritional signals. Growth factors, such as insulin-like growth factor I (IGF-I) and testosterone, are potent anabolic agents, whilst myostatin acts as a negative regulator of muscle mass. Insulin-like growth factor I is unique in being able to stimulate both the proliferation and the differentiation of satellite cells and works as part of an important local repair and adaptive mechanism. Speed of movement, as characterized by maximal velocity of shortening (V_{max}), is regulated primarily by the isoform of myosin heavy chain (MHC) contained within a muscle fibre. Human fibres can express three MHCs: MHC-I, -IIa and -IIx, in order of increasing V_{max} and maximal power output. Training studies suggest that there is a subtle interplay between the MHC-IIa and -IIx isoforms, with the latter being downregulated by activity and upregulated by inactivity. However, switching between the two main isoforms appears to require significant challenges to a muscle. Upregulation of fast gene programs is caused by prolonged disuse, whilst upregulation of slow gene programs appears to require significant and prolonged activity. The potential mechanisms by which alterations in muscle composition are mediated are discussed. The implications in terms of contractile function of altering muscle phenotype are discussed from the single fibre to the whole muscle level.

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The prime mechanical functions of skeletal muscle are to produce force, to generate power and to act as a brake. Skeletal muscle maintains the integrity of our skeleton, allows us to walk, run, jump, talk, eat, breathe and undertake activities essential for life and living. These highly diverse requirements of muscle are met through a number of factors, such as intricate neural control mechanisms, different architectural arrangements, the heterogeneity of fibre types, interplay with series elastic elements, and by the ability to use different sources of fuel to keep the muscle machine working. Yet, as highly diverse as the requirements of muscle are, the mechanism of contraction remains essentially the same throughout the animal kingdom. However, within the basic functional unit of contraction, the sarcomere, there are a multitude of different structural, regulatory and contractile proteins, many of which exist as different isoforms, giving skeletal muscle a multiplicity of isoform expression (Schiaffino & Reggiani, 1996). The ability to increase the number of sarcomeres (i.e. muscle size), together with an ability to alter protein isoform expression, gives muscle the ability to adapt to the different challenges that may be placed upon it. In the context of this review, the term 'plasticity' refers both to changes in muscle size and to changes in protein isoform composition independent of a change in size. This review will focus primarily on information gleaned from studies on human muscle. It attempts to evaluate the mechanisms which regulate the ability of muscle to produce force (i.e. via changes in size) and speed of movement (via changes in myosin isoform expression) and discusses how changes, primarily in physical activity, may alter these properties of muscle. In recent years, developments in

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muscle molecular and cell biology have been applied to the study of human muscle, allowing changes in gene and protein expression to be studied from muscle biopsy samples. This has added to the information gleaned from animal and *in vitro* (cell culture) studies. The focus of this review is on the muscle's contractile machinery *per se* and not on the metabolic adaptations that are known to occur in response to exercise. For these, the reader is referred to other recent reviews (Flück & Hoppeler, 2003; Timmons & Sundberg, 2006).

Force–velocity relationship of muscle

The fundamental mechanical properties of muscle can be described by two relationships elucidated from classic experiments undertaken in the last century, namely the length–tension and force–velocity relationships. The latter provides a particularly suitable basis for studying the plasticity of muscle in the context of its function, and the two extremes of the force–velocity curve provide a convenient framework for discussion. The first, the point at which velocity is zero (i.e. maximal isometric force), denotes the strength of a muscle. Here, other things being equal, force is determined by the physiological cross-sectional area (CSA) of the muscle, or muscle fibre, and is ultimately a reflection of the number of sarcomeres working in parallel with one another. The second, the point at which force is zero, denotes the speed potential of a muscle and is where the velocity of shortening is at a maximum (V_{max}). With all things being equal (specifically temperature and muscle length), V_{max} is ultimately a reflection of the isoform of molecular motor, the myosin cross-bridge contained in the fibre. In adult human muscle, three myosin heavy chain (MHC) isoforms are typically expressed: MHC-I (slow); MHC-IIa (fast); and MHC-IIx (fastest; Fig. 1), although the MHC-IIb gene is present in the human genome. Of course, at each these two extreme points on the force–velocity curve no external power is generated by a muscle. This is not the case during natural movements, where mechanical work is performed and power must be generated. In this instance, power is also a reflection of the curvature of the force–velocity relationship.

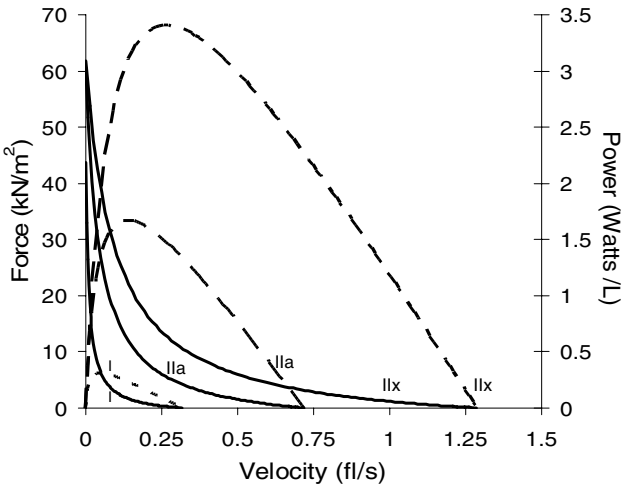

Figure 1. Schematic summary of force–velocity data from human skinned fibres containing MHC-I, MHC-IIa and MHC-IIx isoforms
The data show the slightly greater force per unit area, but markedly greater maximal shortening velocities and power outputs of the MHC-IIa and MHC-IIx fibres. Data were obtained at maximal Ca^{2+} activation and at 12°C. Adapted from Bottinelli *et al.* (1996).

Muscle size

Muscle is by far the largest reserve of protein in the body and therefore has another important role in addition to its mechanical one that being as a dynamic metabolic store. This may be required in extreme situations, such as during times of starvation and critical illness. Most of us are in a state of nitrogen balance, such that muscle mass remains reasonably constant, with an equilibrium existing between the rates of muscle protein synthesis and protein degradation. For a muscle to hypertrophy, there must be a net gain in protein, either from an increase in the rate of protein synthesis or a decrease in the rate of degradation, or both (Rennie *et al.* 2004). Muscle is remarkably sensitive to the mechanical loads placed upon it. Remove the normal mechanical signals that are provided by activities of everyday living, through prolonged bed rest, cast immobilization, spinal cord injury or prolonged exposure to a microgravity environment, and a muscle will atrophy and become weaker. In contrast, overloading a muscle, which is most readily achieved through high-resistance strength-training exercise, increases muscle size and strength. These effects are quite specific to the muscle that has either been disused or loaded. This simple observation provides clues as to the type of local signals needed by a muscle to maintain or increase its size and provides evidence that size regulation cannot solely occur through systemic factors, such as hormones. That is not to say that systemic factors are insignificant, as studies on testosterone have shown. Furthermore, the importance of systemic factors for muscle has recently been demonstrated in studies showing the superior recovery of damaged aged mouse muscles if the older animal shares its circulation with a young animal (Conboy *et al.* 2005). Nevertheless, the general question remains as to how mechanical signals are sensed and interpreted locally as a need for increasing protein synthesis. In recent years, we have begun to unravel the factors regulating these processes.

Rates of protein synthesis may be measured in human muscle through stable isotope techniques, in which labelled amino acid tracers are infused and their

incorporation into muscle biopsy samples is measured (Rennie, 1999). It is clear that exercise, such as high-resistance strength training, initially causes an increase in protein breakdown, which is maintained in the absence of feeding (Phillips *et al.* 1999). However, during recovery and following feeding, muscle protein synthesis increases (Tipton *et al.* 1999), a process which, when repeated over time, results in a net gain in protein and increased muscle mass. Feeding alone increases protein synthesis for 2–3 h after ingestion of amino acids (Tipton *et al.* 1999), but following resistance exercise, protein synthesis may remain elevated for much longer periods (up to 72 h). What are the factors which effect and regulate this process? The creation of new protein initially depends upon the transcription of DNA into mRNA, followed by translation of mRNA into protein. Nutrition, exercise and hormones affect both transcription and translation in muscle. Recent developments in micro-array technology allow experiments to study the responses of many genes simultaneously. Such studies suggest that both strength and endurance exercise change the concentration of many hundreds of mRNAs (Zambon *et al.* 2003; Timmons & Sundberg, 2006), emphasizing that regulation of muscle protein synthesis is complex and involves many factors working in concert with one another. Protein synthesis, or the translation of mRNA, involves the processes of translation initiation, elongation of the peptide chain and termination. These processes are regulated by signal transduction proteins, primarily though phosphorylation and dephosphorylation, and some of the important factors are discussed later. Another essential requirement of hypertrophying muscle fibres is the maintenance of the myonuclear domain. In other words, the number of nuclei must increase so as to maintain the ratio of DNA to protein (Kadi *et al.* 1999*a*). Where do these new nuclei come from?

Satellite cells

Muscle is a relatively stable tissue, in that it is comprised of differentiated muscle fibres containing postmitotic nuclei. Growth and repair of muscle is inextricably linked to the action of a group of myogenic precursor cells, called satellite cells. These cells were initially identified in amphibian muscle by Mauro (1961) and are anatomically distinct from the myonuclei, being located between the basal lamina and the sarcolemma of a muscle fibre. The muscle satellite cell fulfils the basic definition of a stem cell in that it can give rise to a differentiated cell type and can maintain itself by self-renewal (Zammit *et al.* 2006). Upon activation, most, but not all, satellite cells go through a series of stages where they proliferate, differentiate into myoblasts and ultimately fuse with existing myofibres to repair damaged muscle and/or facilitate an increase in its size. Each stage is characterized by the expression of a series of different markers, with satellite cells in the quiescent state being characterized by expression of the cell surface markers CD34 and Pax 7 and the myogenic regulatory (MRF) Myf5. Satellite cell activation is characterized by the rapid onset of MRF, MyoD, with myogenin later marking the commitment to differentiation (Zammit *et al.* 2006). The nuclei of these differentiated cells become incorporated into fibres, may appear centrally following damage and repair, and act as new postmitotic nuclei (see Fig. 2). However, a proportion of the progeny of

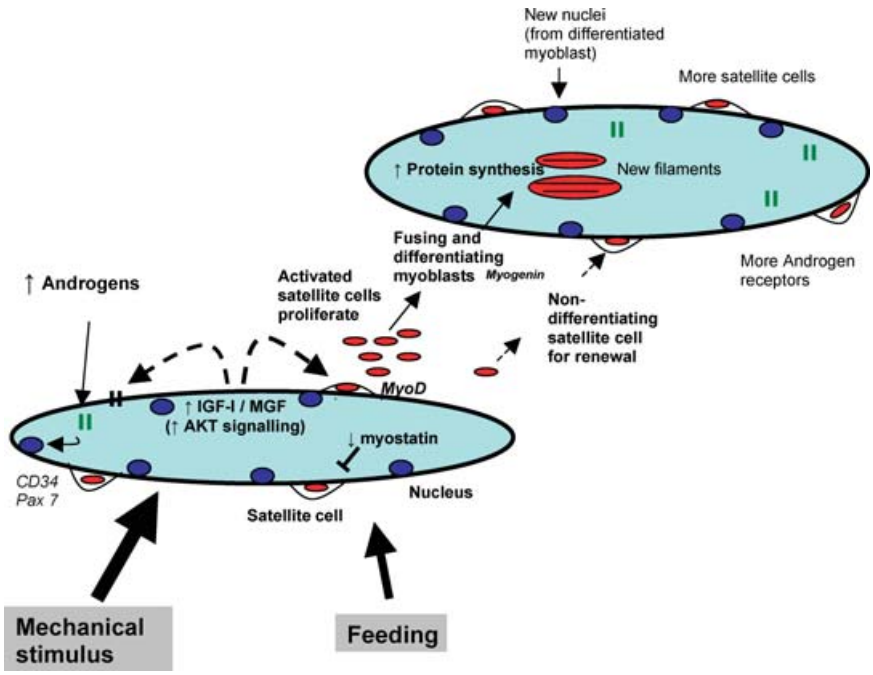

Figure 2. Schematic representation of some key factors influencing hypertrophy of a muscle fibre in response to overload

the activated satellite cells does not differentiate, but withdraws from the cell cycle to replace the activated satellite cell used, in a process of satellite cell renewal (Zammit *et al.* 2006). In extreme cases, such as with Duchenne muscular dystrophy, this cycle of renewal eventually becomes exhausted, resulting in an imbalance between degradation and muscle repair. The skeletal muscles of individuals who have been strength training for several years contain a higher number of satellite cells compared with the muscles of control populations (Kadi *et al.* 1999*a*), and satellite cell number can be increased by strength training in previously untrained populations (Kadi *et al.* 2004). Training studies have shown that significant increases in the proportion of satellite cells can be detected in young men as early as 4 and 8 days after a single bout of maximal exercise (Crameri *et al.* 2004). Furthermore, it seems that satellite cells can respond to a wide range of training intensities, as evidenced by an increase in satellite cell number following endurance cycling exercise in elderly men (Charifi *et al.* 2003), suggesting that damage is not always required for their activation.

The concept of the myonuclear domain in human muscle has been demonstrated in fibres from weightlifting athletes (Kadi *et al.* 1999*b*), where even extremely large fibres (14 000 μm^2) appear to maintain a fixed ratio of fibre size to number of nuclei. It has been suggested that the myonuclear domain is of a size around 2000 μm^2 (Petrella *et al.* 2006). In other words, beyond this a fibre will not be able to hypertrophy unless it can add more myonculei. This concept is supported by a recent study which showed that fibres could hypertrophy without increasing myonuclear number, but only when the fibre size increase was limited to no more than 17% (Kadi *et al.* 2004). This suggests that some 'spare capacity' exists, which allows hypertrophy to occur by increasing protein synthesis, up to the point where the myonuclear domain limit is reached and beyond which addition of new myonuclei is required (Kadi *et al.* 2005). Petrella *et al.* (2006) recently showed that hypertrophy, as determined by the increase in mean fibre area, following 16 weeks of strength-training, was greatest in young men (42% increase) compared with young women (29% increase) and elderly men (16%) and women (34%). This seemed to be as a result of the greater ability of the young male subjects to incorporate more new nuclei into their muscle fibres compared with the other groups. In agreement with the myonuclear domain hypothesis, no differences were observed in the ratio of fibre size to myonuclear number after training in any of the groups studied. Interestingly, in the young female subjects, there appeared to be a lower myonuclear domain in the pretraining muscle when compared with the other subject groups. This was significantly increased with strength training, but only to a level equivalent to that of the other groups (i.e. ~2000 μm^2).

Key regulators of muscle mass

Insulin-like growth factor I (IGF-I). Insulin-like growth factor I is unique in the complex array of growth factors that may act on muscle in that it causes both proliferation and differentiation of satellite cells. IGF-I was originally termed somatomedin and was identified as the factor by which growth hormone (GH) exerted its anabolic actions. Mature IGF-I is a 70-amino-acid peptide, primarily secreted from the liver under the control of GH. The GH–IGF-I axis plays a key role during growth and development, and levels of these hormones reach their peak during adolescence, are maintained at somewhat lower levels during adulthood and then decline in later life. In addition to hepatic IGF-I production, however, it is now well known that other tissues can produce IGF-I for local autocrine and paracrine actions, and some may contribute to circulating IGF-I. For example, Brahm *et al.* (1997) reported a net release of IGF-I from a working muscle into the circulation by measuring femoral arterial and venous differences during isolated knee extension exercise.

Direct infusion of IGF-I locally into the muscles of rats has been shown to cause marked hypertrophy (Adams & McCue, 1998). In human studies, however, indirectly increasing the circulating IGF-I through recombinant GH (rhGH) administration does not result in additional anabolic stimulus to that provided by exercise. Both in untrained young subjects (Yarasheski *et al.* 1992) and in well-trained weightlifting athletes (Yarasheski *et al.* 1993), no increase in the rates of muscle protein synthesis were observed following rhGH treatment over and above those induced by high-resistance strength-training exercise alone. The role of circulating IGF-I remains unclear and needs to be put in the context of the seven IGF-I binding proteins that regulate its bioavailability. Convincing evidence for the greater importance of local IGF-I as opposed to circulating IGF-I in muscle mass regulation was provided by DeVol *et al.* (1990) who studied tenotomy-induced hypertrophy in the soleus and plantaris muscles of rats who had undergone anterior pituitary removal (hypophysectomy), such that GH production was prevented. The findings of this study showed that even in the absence of circulating GH and IGF-I, the muscles still hypertrophied in response to the mechanical overload, with the affected muscles showing a threefold increase in IGF-I mRNA levels.

The anabolic effects of IGF-I have also been clearly demonstrated by numerous cell culture studies, primarily using murine cell lines, in which it has been shown that IGF-I acts to increase the diameter of myotubes, suppress protein degradation, increase amino acid uptake and stimulate protein synthesis (Florini *et al.* 1996; Rommel *et al.* 2001; Bodine *et al.* 2001). More recently, Jacquemin *et al.* (2004) showed similar hypertrophic effects on human muscle cells treated in culture with IGF-I.

IGF-I like growth factor I-treated cells were characterized by increased mean myotube diameter and number of nuclei per myotube, an increase in the fusion index (nuclei incorporated into the myotube) and in MHC content. In human exercise studies, IGF-I mRNA expression in muscles has been found to increase after single bouts of exercise in some studies (Bamman *et al.* 2001; Hameed *et al.* 2003*b*; Kim *et al.* 2005), but not others (e.g. Hameed *et al.* 2003*b*; Psilander *et al.* 2003). Differences between studies may be partly explained by differences in the population under study (young or old), time of biopsy sampling post-exercise, and the IGF-I splice variant (see below) amplified. More consistent increases have been seen following periods of strength training at both the mRNA (Hameed *et al.* 2004; Petrella *et al.* 2006; Kvorning *et al.* 2007) and protein levels (Singh *et al.* 1999).

The autocrine/paracrine role of IGF-I in muscle has more recently been studied with regard to carboxyl terminus extension (E) peptide variants derived from alternative splicing of the IGF-I gene. In both human beings and rats, the IGF-I gene spans more than 70 kilobases and consists of six exons and at least five introns (Stewart & Rotwein, 1996). Two promoters, one adjacent to exon 1 and the other to exon 2, govern gene transcription. The resulting variant mRNA transcripts, with different 5′ untranslated regions and signalling peptides, have been classified as Type/Class 1 (exon 1) and Type/Class 2 (exon 2). In addition to transcription from two promoters, IGF-I is regulated by post-transcriptional events, which yield several mature mRNA transcripts. In all of these variants, exons 3 and 4, which encode the mature 70-amino-acid peptide, are constant, whereas exons 5 and 6 are subject to a complex alternative splicing pattern. Splicing is a complex mechanism by which exons are cut and pasted in different combinations from pre-mRNA. In humans, alternative splicing of IGF-I pre-mRNA leads to the production of three different transcripts at the 3′ end, resulting in different E-peptides. The terms IGF-IEa (skips exon 5, includes exon 6), IGF-IEb (includes exon 5, but skips exon 6) and IGF-IEc (has first 52 bases of exon 5 and includes exon 6) are used here (Hameed *et al.* 2003*a*). It is the IGF-IEc splice variant which has been most closely associated with stretch overload and damage, hence it being termed 'mechano growth factor' (MGF; Yang *et al.* 1996). Effective monoclonal antibodies to the different E peptides, i.e. to IGF-IEa and MGF, have proved somewhat elusive (Shavlakadze *et al.* 2005), so measurement has been restricted to mRNA. However, using a viral construct and a myosin light chain (LC3f) promoter, Barton (2006) recently showed that both the MGF and IGF-IEa gene transfer could increase muscle IGF-I (mature) expression without increasing circulating IGF-I. It is has been proposed that MGF may serve as a 'kick-starting' mechanism in the repair and adaptation processes by activating satellite cells (Hill & Goldspink, 2003). This is supported by the kinetics of MGF and IGF-IEa expression after muscle damage and/or overload, with MGF levels increasing before IGF-IEa (Hameed *et al.* 2003*b*; Petrella *et al.* 2006), which suggests that the two isoforms are differentially regulated, and by cell culture studies where transfection of murine C2C12 cells with MGF expression plasmids increases cell proliferation but inhibits differentiation (Yang & Goldspink, 2002). More recently, Mills *et al.* (2007) have shown that, following treatment with a synthesized 24-amino-acid MGF peptide, satellite cells exhibited significantly enhanced migration *in vitro* (using transwell and invasion assays), as well as exhibiting a more motogenic effect *in vivo*, when administered to mouse muscle. Both IGF-I splice variants possess the same coding region for the mature IGF-I, but because IGF-IEa is expressed at higher concentrations (Hameed *et al.* 2003*b*) it could contribute more to mature IGF-I production and serve to stimulate muscle protein synthesis.

Initial evidence suggested that IGF-I induced hypertrophy via the calcineurin pathway (Musaro *et al.* 1999). However, pharmacological and transgenic blockade of calcineurin signalling do not prevent overload-induced hypertrophy (Bodine *et al.* 2001). Rommel *et al.* (2001) provided evidence that IGF-I served to increase protein synthesis by activating the Akt (also known as PKB)–mTOR (mammalian target of rapamycin) pathway. It is beyond the scope of this review to probe deeply into these complex intracellular signalling events. However, an important study by Baar & Esser (1999) clearly demonstrated that a protein kinase ($p70^{S6k}$) downstream of mTOR is a key regulatory step. The degree of phosphorylation of $p70^{S6k}$ was found to be closely associated with the subsequent muscle growth in overloaded soleus and gastrocnemius muscles of rats.

Testosterone. The androgen testosterone is a steroid hormone synthesized from cholesterol in the Leydig cells of the testes of males and the ovaries of females. Males have circulating values that are 10-fold higher than those found in females and this contributes to the increased muscle bulk in males at the onset of puberty. Endogenous testosterone increases acutely with strength training exercise (Kraemer *et al.* 1990), and strength training also increases the number of androgen receptors (Bamman *et al.* 2001, Roth *et al.* 2003). Testosterone is the basis for the anabolic steroid family of banned substances taken by some athletes. Despite the anecdotal evidence of some athletes and coaches, it is only relatively recently that blinded, placebo-controlled, randomized studies have been conducted to provide some scientific credibility to these claims. For example, Bhasin *et al.* (1996) performed a randomized double-blind study in which 43 young eugonadal males were randomized to receive

placebo, placebo plus strength training, 100 mg week^{-1} testosterone enanthate or 100 mg week^{-1} testosterone enanthate plus strength training. Compared with placebo alone, the testosterone group increased lean body mass, quadriceps CSA as determined by magnetic resonance imaging (MRI) and the weight lifted during a single squat. The changes due to testosterone were similar to those evoked by exercise alone. However, when testosterone administration was combined with resistance exercise, the change in all three parameters was greater than the changes resulting from exercise alone. Subsequently, Sinha-Hikim *et al.* (2002) investigated the effects of 20 weeks of testosterone enanthate administered at 125, 300 or 600 mg week^{-1} and reported that fibre size increased in a dose-dependent manner. Analysis of the biopsy samples showed that both myonuclei and satellite cell number also increased in a dose-dependent manner up to a dose of 600 mg week^{-1}. *In vivo* binding studies suggest that the androgen receptors in most tissues are either saturated or downregulated at testosterone concentrations at the lower end of the normal range. However, as mentioned earlier, exercise training has been shown to increase the number of androgen receptors. This is important because athletes may take much higher doses than that used in the study by Bhasin and colleagues. It is also possible that the supraphysiological doses evoke hypertrophy through a non-androgen-receptor-mediated mechanism (Solomon & Bouloux, 2006). For instance, Urban *et al.* (1995) reported that administration of testosterone to elderly men (so that their circulating levels were equivalent to those of young males) increased muscle strength and increased rates of muscle protein synthesis. Furthermore, the mRNA concentration of IGF-I was increased, leading the authors to conclude that the testosterone-induced increase in protein synthesis might be mediated by the IGF-I system. In contrast, lowering circulating testosterone levels by administration of a gonadatrophin-releasing hormone (GnRH) agonist is associated with a reduction in fractional rates of muscle protein synthesis (Mauras *et al.* 1998). In an interesting approach, Kvorning *et al.* (2006) performed a strength-training study in which testicular production of testosterone was blocked through inhibition of pituitary-secreted lutenizing hormone by the administration of goserelin (a GnRH analogue). This had the effect of significantly ablating the hypertrophic response to strength training. However, it was without effect on the other factors that are likely to contribute to hypertrophy, such as IGF-IEa and MGF (Kvorning *et al.* 2007). The mechanism of action of testosterone on satellite cells is not clear, but androgen receptors have been identified in satellite cells (Sinha-Hikim *et al.* 2003). It is possible that testosterone might promote entry of the cells into the cell cycle, or that androgens might promote differentiation. These events are those which are also believed to be regulated in part by IGF-I. As with GH/IGF, a progressive decline in circulating testosterone levels (andropause) is associated with ageing. However, testosterone replacement in elderly hypogonadal men has produced rather inconsistent and modest increases in muscle mass and strength and is associated with a number of side-effects, such as fluid retention, gynaecomastia, polycythaemia and acceleration of benign or malignant prostatic tumours (Borst, 2004). The illegal use of anabolic steroids continues in sport and body building. A perusal of related websites reveals the large number of testosterone derivates that are available and the variety of manipulations and practices (e.g. 'stacking') that are purported to maximize their effects. Credible scientific data are lacking to support many of these claims.

Myostatin. Myostatin (growth differentiation factor 8) was identified as a negative regulator of muscle mass following studies undertaken on the Belgian Blue and Piedmontese breeds of cattle (McPherron & Lee, 1997). These animals have a markedly hypertrophied or 'double-muscled' phenotype associated with a mutation in their myostatin gene. Subsequent experiments on myostatin knockout mice (McPherron *et al.* 1997) confirmed that the hypertrophied phenotype is a response to a myostatin gene mutation. A recent case report of a young child exhibiting gross muscle hypertrophy revealed the child to have a mutation in the myostatin gene (Schuelke *et al.* 2004). In contrast to IGF-I and testosterone, which stimulate protein synthesis, myostatin is a negative regulator of muscle growth and signals via Smad transcription factors. It controls cell cycle progression in a manner opposite to that of IGF-I–phosphatidyl inositol 3-kinase (PI3k)–AKT signalling, by inhibiting satellite cell proliferation. Recent human exercise studies have shown that myostatin is downregulated with strength-training exercise (Roth *et al.* 2003; Kim *et al.* 2005). The potential therapeutic benefits of increasing muscle mass through myostatin inhibition have been realized, and there are currently clinical trials underway. However, whilst clear evidence exists for the downregulation of myostatin having the effect of increasing muscle mass, there is also evidence to show that the quality of muscle, in terms of its function, may be poor. Amthor *et al.* (2007) have recently shown that although the muscles of myostatin knockout mice are twice as large, they have a significantly lower specific force, meaning that these highly hypertrophied muscles are intrinsically weaker.

Cortisol, atrogin and muscle atrophy. Thus far the focus has been on factors which regulate increases in muscle size. Whilst overload promotes hypertrophy, disuse promotes the atrophy of muscle. Cortisol is the primary glucocorticoid secreted by the adrenal glands. Among its actions, cortisol promotes the breakdown of amino

acids, delivering them to the liver for synthesis to glucose via gluconeogenesis. Glucocorticoids have been used to induce muscle atrophy and to study the mechanisms which regulate muscle protein degradation. For example, the synthetic glucocorticoid dexamethasone induces atrophy and evokes a number of parallel changes in muscle gene expression which have been termed 'atrogenes'. The gene most dramatically induced is a Ubiquitin-ligase (E3), called atrogin 1 (MAFbx), which is a muscle-specific F box protein that is induced many-fold in fasting, diabetes and cancer (Sacheck *et al.* 2004). In addition, another muscle-specific E3, MuRF1, is also highly induced in atrophying muscle (Bodine *et al.* 2001). Both of these genes have been shown to be upregulated in human muscle atrophy induced by lower limb immobilization (Jones *et al.* 2004). Interestingly, administration of glucocorticoids together with IGF-I to rats significantly attenuates the glucocorticoid-induced muscle atrophy and myofibrillar protein breakdown (Kanda *et al.* 1999). Similar studies on cells in culture (Sacheck *et al.* 2004; Sandri *et al.* 2004) have shown that this effect is likely to be mediated by the rapid suppression of atrogin-1 by IGF-I and indeed insulin, such that only when insulin is low (such as during fasting) are the catabolic effects of glucocorticoids on muscle evident (Wing & Goldberg, 1993). The mechanism seems to involve the Forkhead box O (Foxo) class of transcription factors, and Foxo gene expression is induced by glucocorticoids. Akt blocks the function of Foxo transcription factors by phosphorylation, leading to their sequestration in the cytoplasm away from their target genes. Thus, when phosphorylated by the action of IGF-I, Akt impairs the action of Foxo. However, without IGF-I-induced activation of Akt, dephosphorylation of Foxo factors leads to nuclear entry and protein degradation (see Sandri *et al.* 2004). Furthermore, glucocorticoid administration has also been shown to upregulate myostatin expression both *in vitro* and in human skeletal muscle (Ma *et al.* 2003).

Functional implications of increasing muscle size

Muscle strength can be defined as the maximal amount of isometric force that can be generated during a single maximal voluntary contraction. Relative to data obtained from whole animal muscle, or from single fibres studied *in vitro*, interpreting the measured force or torque output produced from contractions made *in vivo* presents a large number of challenges. These challenges include isolation and fixation of muscles or muscle groups under study, as well as the level of muscle activation, both intracelleular and neural. The latter can be assessed using twitch interpolation techniques, through direct electrical stimulation of the muscle belly or indirect stimulation of the nerve. This is important because it is clear that much of the early adaptation to strength training does not result from an increase in muscle size but from improved activation of the muscle. That said, is interesting in this regard that in the study by Phillips *et al.* (1999) the exercise-induced increase in muscle protein synthesis was greater in untrained individuals compared with trained individuals, suggesting the possibility of a faster rate of growth in untrained individuals. However, even when appropriate measurements have been made, relating mechanical properties of a muscle to its architecture is not trivial. Modern developments in both MRI and ultrasound imaging have proved extremely useful in allowing muscle

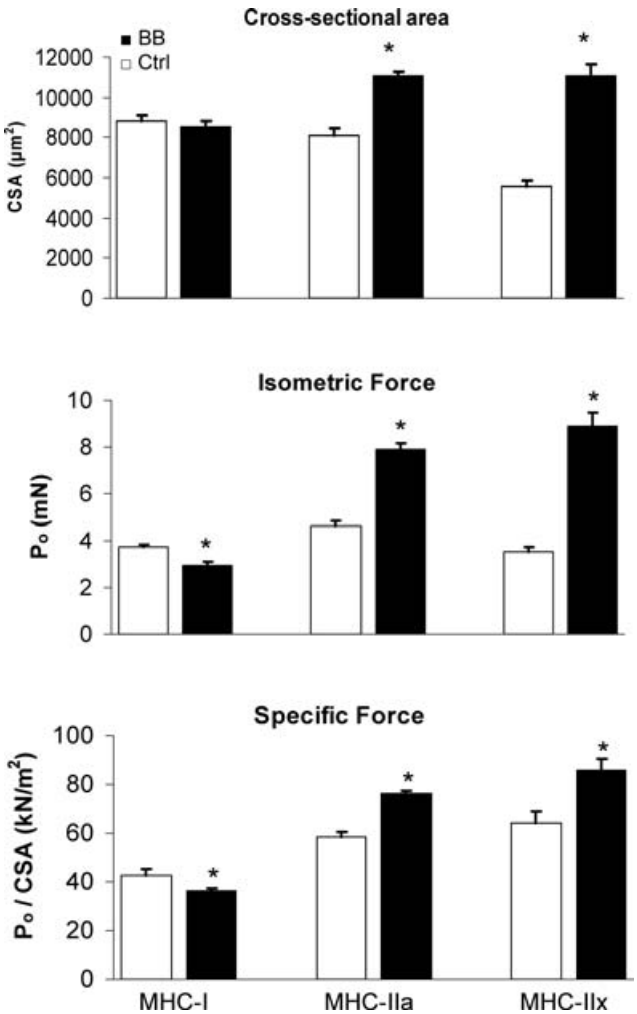

Figure 3. Data from single muscle fibre experiments comparing maximal isometric force (P_o), cross-sectional area and specific force in MHC-I, -IIa and -IIx fibres from the muscles of body builders (BB) and control subjects (Ctrl)
The body builders exhibit similarly sized, but weaker MHC-I fibres, but highly hypertrophied and intrinsically stronger MHC-IIa and -IIx fibres. Data adapted from D'Antona *et al.* (2006). *BB Significantly different from ctrl ($P < 0.05$)

function to be expressed in terms of its functional anatomy. Whilst MRI provides good resolution of anatomical cross-sectional area of muscle, allowing for distinction between muscle, fat and connective tissue, this does not tell us anything about the alignment of the muscle fibres. Here developments in ultrasound imaging allow fascicles to be identified and measured in terms of their lengths and their angles of pennation (see Narici & Maganaris, 2006). Following strength training, increases in the region of 10% may be expected in terms of anatomical cross-sectional area of the quadriceps, as determined by MRI (Aagaard *et al.* 2001). However, the functional changes are confounded by changes in muscle architecture. In this particular study, the pennation angle of the fibres in the vastus lateralis increased from 8 to 11 deg following training. Whilst this serves to increase the physiological cross-sectional area of the muscle and thus force potential, the increase in angle of pull decreases the effectiveness of force applied by each fibre to the tendon. Thus, any gains in strength represent a balance of these two processes.

The last 15 years have seen exploitation of *in vitro* studies of human muscle at the level of the single fibre which overcome many of the confounding factors to *in vivo* measurements as discussed above. Single fibre segments can be isolated from biopsy samples and chemically skinned to remove the sarcolemma. These permeablized fibres can be activated chemically through immersion in a solution with a high concentration of calcium, usually at 12 or 15°C. Studies have consistently shown a higher force per unit area for MHC-IIa and -IIx fibres compared with MHC-I fibres (Bottinelli *et al.* 1996). Cross-sectional

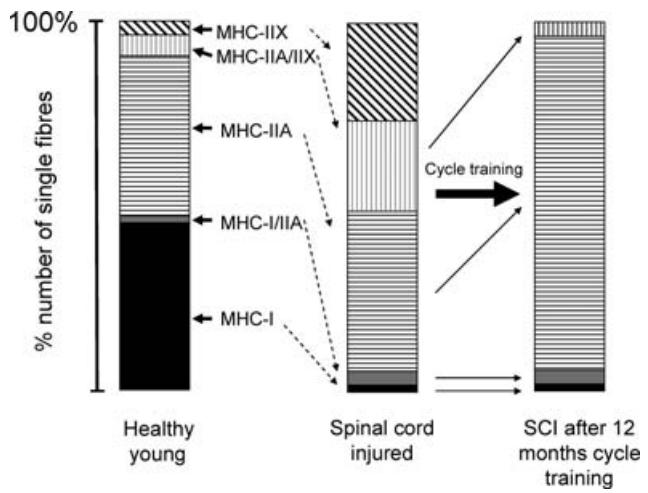

Figure 4. Distribution of MHC isoform expression in human single fibres obtained from the vastus lateralis as determined by gel electrophoresis
The first column shows data from a healthy young population, the second from a spinal-cord-injured (SCI) population and the third from the same spinal-cord-injured subjects following 1 year of electrically evoked cycle training. Adapted from Andersen *et al.* (1994, 1996).

(Shoepe *et al.* 2003) and longitudinal (Widrick *et al.* 2002) single fibre studies following strength training in young individuals suggest that fibre force increases in proportion to fibre CSA. This, coupled with the apparently greater sensitivity to hypertrophy of the type II fibres, would provide a mechanism for increasing force per unit area in a whole mixed muscle after training. This phenomenon is enhanced in the extreme case of highly hypertrophied muscles from body builders. The MHC-IIa and IIx fibres were not only significantly larger, but also had a significantly higher force per unit compared to the control subjects (Fig. 3). Interestingly, the MHC-I fibres in the body builders were of similar size, but were slightly weaker (D' Antona *et al.* 2006).

Myosin isoforms and speed of movement

Skeletal muscle myosins are class II myosin proteins. Each myosin molecule (~500 kDa) is a hexamere comprised of two heavy chains and four light chains which are assembled to form two globular heads with a long tail. Studies on chemically skinned single human fibres have shown that, as with rodent muscle, it is the MHC isoform which is the prime determinant of V_{max}, the velocity at which peak power occurs (Bottinelli *et al.* 1996; Fig. 1), as well as the rate of force development (Harridge *et al.* 1996) in a fully active fibre. As mentioned earlier, in normal adult human skeletal muscle fibres, three MHC isoforms may be expressed: one slow, the MHC-I (β-slow), and two fast, MHC-IIa and MHC-IIx. The latter is the fastest myosin and is synonymous with the MHC-IIb and MHC-IId isoforms found in older literature. Fibres may contain more than one MHC isoform (Fig. 4), a phenomenon which is more frequent in the muscles of older people (Klitgaard *et al.* 1990*b*). These hybrid fibres may represent fibres that are transforming from one type to another. Prediction of the direction of change may be made by comparing the relative MHC isoform content with MHC mRNA expression in the same fibre (Andersen & Schiaffino, 1997).

In addition to the heavy chain components, the S1 region of the cross-bridge also contains two pairs of myosin light chains (MLC, regulatory and essential). These MLCs also exist as fast and slow isoforms, which together with fast and slow isoforms of troponin, tropomyosin and other proteins, provide a potential multiplicity of contractile and regulatory protein isoform expression in muscle (Schiaffino & Reggiani, 1996). In rat muscle fibres, the MLC3f isoform serves to fine-tune V_{max}, and its expression seems to explain differences in V_{max} between fibres which contain the same MHC isoform (Bottinelli *et al.* 2004). However, in human muscle fibres such a regulatory role has yet to be clearly established.

Analysis of human muscles with different functional demands reveals a selective distribution of fibre types,

with the postural soleus muscle being dominated by slow MHC-I-containing fibres and the triceps brachii of the arm being dominated by fast MHC isoforms (Harridge *et al.* 1996). Cross-sectional studies of muscles from athletes specializing and excelling in different speed and endurance events have long since highlighted the importance of fibre type distribution in relationship to athletic performance. Costill *et al.* (1976) first showed that the gastrocnemius muscle of top sprint athletes was dominated by fast type II fibres and that of top endurance athletes by slow type I fibres. Exercise physiologists have since struggled to explain the extent to which this phenomenon is the result of natural endowment, specific training regimens, or a perhaps a combination of both. The classic cross-innervation experiments in cats undertaken by Buller *et al.* (1960) provided the first real evidence that the mechanical characteristics of a muscle could be altered by changing the type of neural input a muscle received. This was later shown to be due to a change in protein isoform composition.

Slow-to-fast transformations in human muscle

Sprinting and weightlifting are sporting activities which require the generation of high power outputs. Similarly, undertaking simple activities of daily living, such as rising from a chair or climbing onto a bus, also requires the generation of relatively high power outputs. These activities are particularly compromised in elderly people, both by a loss of muscle mass (sarcopenia) and by a relatively greater loss of MHC-II isoforms, resulting from the preferential atrophy of type II fibres (Klitgaard *et al.* 1990*a*). A switch towards MHC-II and, particularly, MHC-IIx isoforms would be of use for maximizing explosive power output, at least in the short term. During muscle contraction, fast MHC-II fibres/motor units are recruited after slow motor units in a size-ordered manner and tend to fire in intermittent high-frequency bursts. However, patterns of activity which recruit motor units in a fast-phasic manner, and which might be expected preferentially to upregulate fast isoforms (such as through sprint, power and weight training), appear to have the opposite effect, in that they induce a downregulation of MHC-IIx isoforms (see Andersen *et al.* 2000). Only in dennervated rat muscle is there some evidence of a slow-to-fast transformation evoked through intermittent bursts of high-frequency (150 Hz) stimulation, but the effects of the dennervation itself may be confounding Gorza *et al.* (1988). The observation that MHC-IIx isoforms are downregulated with increased activity has been reasonably consistent in a large number of studies and suggests a subtle regulation of the two fast isoforms. This is supported by the rapidity with which MHC-IIx mRNA expression is downregulated, just hours/days after a single bout of exercise (J. L. Andersen, unpublished observation) and by the return to above-pretraining levels following the cessation of training and the resumption of a sedentary life style (Andersen & Aagaard, 2000). As the final point suggests, it is in fact disuse which has the effect of causing a slow-to-fast transformation in MHC expression. Figure 4 shows the distribution of MHC isoforms from the electrophoretic separation of MHC isoforms in single fibres obtained from the vastus lateralis muscle of healthy young subjects and from individuals having sustained a spinal cord injury (SCI) some years earlier. In contrast to the healthy subjects, in whom approximately 50% of fibres contain the MHC-I isoform, the SCI subjects have almost no fibres containing the MHC-I isoform. Their muscles are dominated by fibres expressing MHC-II isoforms, with more than half of the fibres expressing MHC-IIx either alone, or in combination, with MHC-IIa isoforms. Whilst these are potentially very fast muscles, the accompanying atrophy negates any benefit of such a slow-to-fast switch in terms of function. The reason for this phenomenon is unclear, but it has been suggested that the MHC-IIx is the default form of the protein and the most efficient for storage (Goldspink *et al.* 1991).

Fast-to-slow transformations in human muscle

Following on from the re-innervation experiments of Buller *et al.* (1960), Salmons & Vrbová (1969) demonstrated in rabbit muscle that a fast-to-slow transformation in the contractile properties of muscle could also be brought about by electrically stimulating the nerve with a frequency pattern which is normally delivered to a slow muscle. Since then, numerous animal studies have used the chronic low-frequency (e.g. 10 Hz) stimulation model to induce change in fast muscles towards the slow oxidative type (Pette, 2006). These studies have shown that this alteration is a phased process, with transitions of various systems following different time courses, including changes in metabolism, Ca^{2+} handling kinetics and changes in the contractile proteins. The change in MHC isoform expression in rodents also goes through a phased process (MHC-IIb ↔ MHC-IIx ↔ MHC-IIa ↔ MHC-I). It is worth noting here that the responsiveness of these animal muscles can be highly species dependent (with rat muscles more resistant than rabbit muscles to switching) and indeed strain dependent within species (Pette & Vrbová, 1992). Based on these animal studies, some human muscles have undergone electrical stimulation conditioning for therapeutic purposes. These include conditioning the latissimus dorsi for cardiac assist and the gracilis for use as an anal sphincter. The prime task of these conditioning programmes is to increase fatigue resistance; this can be achieved with metabolic adaptations which do not necessarily alter MHC isoform composition.

What advantages might a slower myosin isoform itself confer? The traditional view is that type I muscles are more efficient. For instance, it has been shown that during cycling, endurance athletes with a higher percentage of type I fibres in the quadriceps muscle are more efficient (i.e. lower metabolic cost) than those with a lower percentage of type I fibres (Coyle *et al.* 1992). *In vitro* studies using human single skinned fibres provide a more direct measure of the efficiency of the myosin molecule *per se*, but the studies are somewhat contradictory. Measuring ATPase activity during isometric contractions, Stienen *et al.* (1996) showed that the isometric tension cost of MHC-I fibres is less than half that of the type MHC-IIx fibres. However, using a fluorescently labelled phosphate binding protein to calculate ATP use during isovelocity shortening, He *et al.* (2000) reported no difference in efficiency during shortening between MHC-I and MHC-IIa fibres.

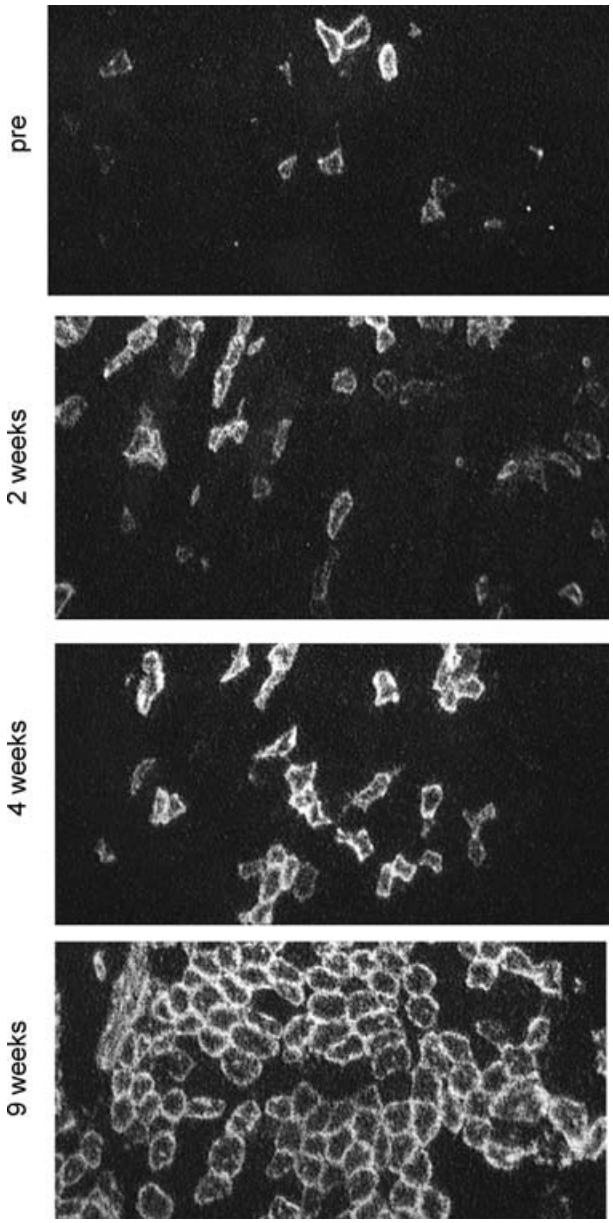

Figure 5. Sections of a muscle biopsies processed with *in situ* hybridization with ^{35}S-labelled probes for MHC-I mRNA. Samples were taken from the tibialis anterior muscle of a spinal cord injured subject prior to, and during 9 weeks of electrical stimulation training and show an increase in fibres expressing MHC-I mRNA
From Harridge *et al.* (2002), with permission.

The use of chronic low-frequency electrical stimulation approaches in animals has proved a useful model for studying molecular mechanisms underlying fibre switching, but the physiological relevance of such extreme animal models has been questioned. The animal studies which have been equivalent to human training studies have been far less effective in transforming fibre types than electrical stimulation models (e.g. Green *et al.* 1984). In one of the earlier human training studies Andersen & Henriksson, 1975), subjects performed 8 weeks of endurance cycling training, which resulted in no increase in the proportion of type I, but a decrease in the proportion of type IIb(x) fibres, as they apparently converted to type IIa fibres. In a longer study, which involved 24 weeks of 45 min of cross-country skiing three times per week, no increase in the proportion of type I fibres was again observed (Ingjer, 1979). There are many challenges to performing training studies in human subjects, ranging from the heterogeneity of the training population to the adherence to the training regimen, as well as any additional activity which may be undertaken at a detriment to the desired protocol. Spinal-cord-injured subjects provide a useful model for studying the plasticity of human muscle in that their muscles may be electrically stimulated in a reasonably standardized manner. This is similar to the chronic electrical stimulation protocols used in animal experiments. One such study reported that a year of electrically evoked cycle training (30 min day^{-1}, 3 times per week) caused a reduction in MHC-IIx isoform expression, but no increase in the number of fibres containing MHC-I isoforms (Andersen *et al.* 1996; Fig. 4). However, the training duration for which the quadriceps muscles were activated represented less than 1% of the week. Thus, the disuse signal (favouring a slow-to-fast switch) is still likely to be highly influential. In a subsequent study, the tibialis anterior muscle was stimulated for 4 and, in some instances, 9 weeks for 2 h day^{-1} progressing up to 6 h day^{-1} each weekday, under isometric loading conditions (Harridge *et al.* 2002). This led to a dramatic increase in muscle fatigue resistance, but failed to increase the relative proportion of MHC-I isoforms. However, an increase in the number of fibres expressing MHC-I mRNA was identified using *in situ* hybridization (Fig. 5). Thus, at least at the level of gene transcription, a switch towards a slow muscle phenotype had been initiated by this protocol.

Mechanism of fibre switching

The mechanisms involved in fibre switching must ultimately involve the repression of one set of genes and the upregulation of another. A key role in the upregulation of the slow-fibre genes has been proposed for a Ca^{2+}-mediated signalling cascade by a calcineurin-regulated mechanism (Chin *et al.* 1998). These workers found that in rats, when the calcineurin pathway was inhibited by cyclosporin A, the number of fast fibres in the soleus doubled. Their hypothesis was that prolonged increases in intracellular Ca^{2+}, through prolonged low-frequency stimulation, activated the calcineurin pathway. Calcineurin causes the dephosphorlyation of the transcription factor NFAT (nuclear factor of activated T cells). In the non-phosphorylated state, NFAT is able to translocate to the nucleus where it initiates the slow fibre program through activation of the myocyte enhancer factor (MEF2) family of transcription factors. Intermittent Ca^{2+} activation (high-frequency bursts), in contrast, does not result in calcineurin activation and NFAT remains phosphorylated and does not translocate to the nucleus, thus the fast/default program prevails. Whilst this elegant study was convincing, a subsequent study by Murgia *et al.* (2000) showed that slow genes could also be upregulated by the extracellular signal-regulated kinase 1/2 (ERK 1/2) pathway. Furthermore, overexpression of peroxisome proliferators-activated receptor-γ coactivator 1α (PGC 1α, the so-called master regulator of mitochondrial biogenesis) also leads to mouse muscles being dominated by red, type I fibres, suggesting that in addition to regulating mitochondrial biogenesis it also controls other genes that are part of the slower muscle phenotype (Lin *et al.* 2002). Thus, as with muscle growth, a multitude of factors are likely to act in concert to regulate switching.

What genes are preferentially expressed in type I fibres? The family of myogenic transcription factors in general, and members of the MRF family of basic helix–loop–helix transcription factor proteins in particular, have been suggested to play an important role in the differentiation processes of the adult skeletal muscle cells through transcriptional control of phenotype-specific proteins. In rats, MyoD mRNA has been shown to be most prevalent in fast glycolytic muscles, whereas myogenin mRNA has been shown to be most prevalent in slow oxidative muscles, and this relationship followed phenotype transition caused by cross-innervation (Hughes *et al.* 1993). These results lead to the suggestion that MyoD and myogenin control fast and slow fibre-type-specific gene expression, respectively. It has also been suggested that different collaborative units of transcription factors, including the MEF-2 family of transcription factors, might exert transcriptional control of functionally related (and perhaps phenotype-specific) subsets of genes. In agreement with the above, myogenin mRNA was found to be upregulated (after 2 weeks) in the tibialis anterior muscle of SCI subjects which had been subjected to chronic low-frequency electrical stimulation training, preceding the increase in MHC-I mRNA (Harridge *et al.* 2002; Vissing *et al.* 2005). However, contrary to what might be expected, MyoD mRNA also increased, although later than myogenin. The mRNA levels of the other myogenic regulatory factor family members, myogenic factor 5 (Myf5) and myogenic regulatory factor 4 (MRF4), and the MEF family members MEF-2A and MEF-2C did not change significantly in this study. Taken together, these observations suggest that myogenin is indeed involved in the regulation of the slow oxidative phenotype in human skeletal muscle fibres, but MyoD would appear to have a more complex regulatory function. However, it needs to be noted that this study was performed on muscle from spinal-cord-injured patients and not muscle from healthy subjects. Additional studies are required to elucidate these mechanisms in human muscle further.

Functional implications of myosin switching

For over 30 years attempts have been made to correlate the dynamic function of whole muscle with analysis of biopsy samples of its composition. These have only met with moderate success even when electrophoretically separated MHC isoforms were related to angle-specific torque–velocity properties generated by involuntary, electrically evoked contractions (Harridge *et al.* 1995). Complications of *in vivo* measurement of isometric force were described earlier. During dynamic contractions these measurements are further confounded by the role of series elastic elements (tendons). Ideally, for objective measurements to me made, both the mechanical advantage of a joint and the length of the muscle at which the force or torque is measured must be standardized throughout the range of velocities studied. However, during contraction of a muscle–tendon complex, tendon extension is directly related to the force applied by the muscle fibres. This in turn depends upon the speed at which they are shortening (the force–velocity relationship). This means that during shortening of a muscle–tendon complex, muscle length itself will vary depending upon the velocity of contraction (Ichinose *et al.* 2000). This fact, coupled with changes in pennation angle during contraction, make *in vivo* muscle function, in terms of fibre shortening, difficult to interpret.

Nevertheless, increases in muscle power as a result of hypertrophy causing an increase in the force component of the force–velocity relationship are well documented. In contrast, a sideways shift in the force–velocity relationship would be expected on the basis of a change in muscle composition. This is more difficult to detect, particularly since changes in activation or tendon compliance with training may superimpose themselves on any mechanical changes induced by a shift in muscle composition. A

left-to-right shift might be expected on the basis of there being relatively more MHC-II isoforms, resulting not necessarily from fibre switching *per se*, but from the selective of hypertrophy of type II fibres with training. Nevertheless, Andersen *et al.* (2005) reported an increase in the maximal angular velocity of externally unloaded leg extension following a period of detraining, itself following 12 weeks of strength training. This was despite a fall in isometric torque to pretraining levels, and may be partly explained by the increase in MHC-IIx isoform expression which accompanied the detraining period.

The skinned fibre preparation, using either load clamps to obtain force–velocity data or slack test manoeuvres for direct determination of V_{max} (V_o), has once again been used to overcome some of the difficulties encountered in the interpretion of *in vivo* function changes. These studies have confirmed the dominance of MHC isoform expression in regulating V_o, but have also shown that it is possible to alter V_o in fibres expressing the same MHC isoform. One example is the increase in V_o measured in MHC-I fibres from the soleus muscle following 17 days of space flight (Widrick *et al.* 1999). Strength (Widrick *et al.* 2002) and sprint (Harridge *et al.* 1998) training have been found not to affect V_o. In elderly men, however, Trappe *et al.* (2000) reported an increase in V_o in both MHC-I and MHC-IIa fibres as a result of 12 weeks of strength training. This could not be explained by a change in MLC composition, leaving other possible causes to include some form of change in the structural arrangement of the sarcomere, or a change in the behaviour of the cross-bridge itself. Recently, the latter was investigated using the *in vitro* motility assay, where the motility of fluorescent actin filaments running on a bed of myosin was measured using myosin extracted from single MHC-I and MHC-IIa fibres pre- or post-training (Canepari *et al.* 2005). This study showed that 12 weeks of strength training increased the speed of actin motility on myosin extracted from MHC-IIa, but not MHC-I fibres, in both young and elderly subjects. This suggests a post-translational alteration of the myosin as a result of the strength-training intervention. Glycation, for example, has previously been linked with a slowing of motility in myosins extracted from aged muscle fibres (Höök *et al.* 1999).

Conclusion

As in many other species, human skeletal muscle is a highly plastic tissue, being able to adapt its size and protein isoform expression to changes in usage. Developments in molecular and cell biology have, over the last couple of decades, helped us to understand more fully the mechanisms regulating these processes. In parallel, developments in imaging have improved our understanding of muscle structure and function *in vivo*, whilst the application of *in vitro* approaches, particularly the skinned fibre technique, have allowed the plasticity of human muscle to be studied in the context of its function.

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