

## REVIEW

# Modifying muscle mass – the endocrine perspective

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### Abstract

This review describes the major hormonal factors that determine the balance between human skeletal muscle anabolism and catabolism in health and disease, with specific reference to age-related muscle loss (sarcopenia). The molecular mechanisms associated with muscle hypertrophy are described, and the central role of the satellite cell highlighted. The biological dynamics of satellite cells, varying between states of quiescence, proliferation and differentiation are strongly influenced by local endocrine factors. The molecular mechanisms of muscle atrophy are examined focussing on the causes of sarcopenia and associations with systemic medical disorders. In addition, evidence is provided that the mechanisms

of atrophy and hypertrophy are unlikely to be simple opposites. Novel endocrine mechanisms underpinning mechano-transduction include IGF-I subtypes that may differentiate between endocrine and mechanical signals; their interaction with classical endocrine factors is an active area of translational research. Recently acquired knowledge on the mechanism of anabolic effects of androgens is also reviewed. The increasingly recognised role of myostatin, a negative regulator of muscle function, is described, as well as its potential as a therapeutic target. Strategies to counter age-related sarcopenia thus represent an exciting field of future investigation.

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### Introduction

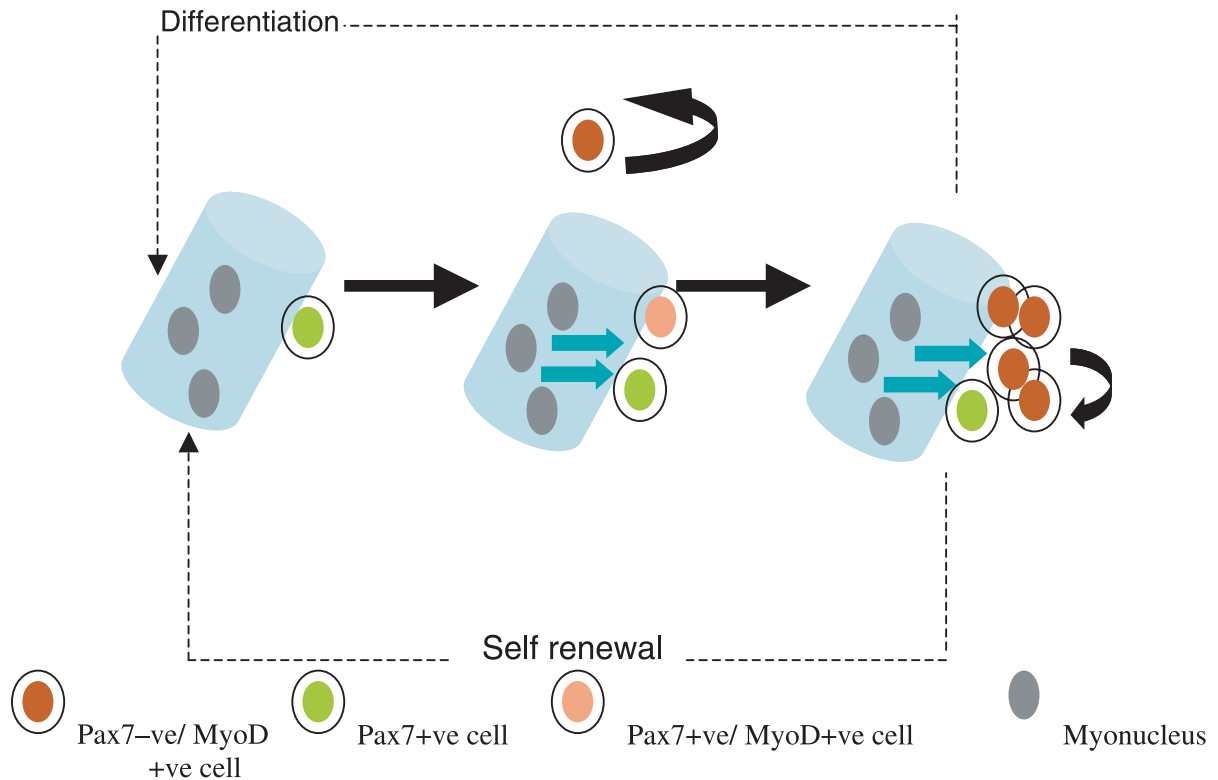
Endocrine factors influence muscle growth and development throughout life and states of hormonal excess or deficiency adversely affect both muscle structure and function (Veldhuis *et al.* 2005). This review examines some of the key endocrine factors that affect the dynamic balance between anabolic and catabolic stimuli in muscle, which are now known not to be simple opposites (Glass 2003).

Muscle cells function as a syncytium, with several nucleated cells fusing to form the muscle fibre – the key cellular activator of this process being the satellite cell (Wozniak *et al.* 2005). A sequence of transcription factors act on satellite cells – the myogenic regulatory factors (MRFs) – comprising myogenin, MRF4, MyoD and Myf5, which are expressed in an anatomical and time-dependent manner (Buckingham *et al.* 2003).

Satellite cells are located beneath the basal lamina of muscle fibres and are a distinct muscle cell subtype responsible for post-natal adaptation, growth and repair (Mauro 1961). They have a variety of potential fates, including proliferation, fusion or trans-differentiation (Zammit *et al.* 2004, Fig. 1). Increasingly, observations are being made of stem cells adopting satellite cell status or vice versa – either locally derived, as muscle-derived stem cells (Mourkioti & Rosenthal 2005, Sinanan *et al.* 2004)

or of bone marrow origin (Chen & Goldhamer 2003). Our present understanding of the factors affecting satellite cell biology has become increasingly sophisticated – of special interest are the influence of mechanical stimulation, ageing and endocrine factors (Dhawan & Rando 2005, Wagers & Conboy 2005, Wozniak *et al.* 2005, Scime & Rudnicki 2006, Sherwood & Wagers 2006).

*In vivo*, satellite cells may be activated following a single episode of high-intensity exercise (Cramer *et al.* 2004), although this is insufficient to induce terminal differentiation. This response is attenuated during ageing (Gallegly *et al.* 2004), the mechanism of which is unclear – although *in vitro*, insulin-like growth factor-I (IGF-I) appears to extend proliferative capacity and delay cellular senescence (Mouly *et al.* 2005). Reduced numbers of satellite cells in ageing may also be associated with reduced chromosomal telomeric length (Wernig *et al.* 2005). In addition, prolonged atrophy itself induces a reversible dysfunction of satellite cells (Mitchell & Pavlath 2004). Recent reports have incriminated the Notch signalling pathway in sarcopenia of ageing. In elegant parabiotic experiments, younger and older animals' circulations were exchanged, with the Notch pathway appearing to be reactivated in older mice exposed to 'young' serum, suggesting a role for circulating endocrine factors (Conboy *et al.* 2003, 2005).



**Figure 1** Adapted from Zammit *et al.* (2004). A diagram showing a variety of satellite cell fates. Quiescent satellite cells (green) activate to co-express Pax7 and MyoD (orange), and then may downregulate Pax7, maintain MyoD (red), proliferate and/or differentiate. The arising progeny may become quiescent, thus renewing the satellite cell pool.

Muscle fibre type is a further level of skeletal muscle anatomy responsive to exercise and endocrine factors. Traditional classification has comprised slow-twitch (type I) and fast-twitch (type II) fibres further subdivided into type IIa and type IIb according to energy utilisation (although type IIb in humans is somewhat homologous to a further type IIx found in rodents). Testosterone induces hypertrophy of both type I and type II fibres (Kadi *et al.* 1999). There appear to be significant anatomical site, age and gender-specific differences in muscle fibre-type responses to resistance training in humans, the precise endocrine correlates of these observations are, as yet, not fully elucidated (Martel *et al.* 2006).

### Sarcopenia

The term sarcopenia (the loss of muscle mass and strength during ageing) is Greek-derived meaning 'lack of flesh', and may co-exist with osteopenia/osteoporosis. Measurement and clinical definition have not reached international consensus (Lauretani *et al.* 2003). The mechanism of sarcopenia is incompletely understood; hypotheses include age-related dynamics in IGF-I, its splice variants and impaired mechano-transduction (Goldspink & Harridge 2004).

A recent report using microarray analysis comparing muscle biopsies in 10 young versus 12 older subjects found that muscle from older subjects demonstrated differences associated with apoptosis, such as increased complement component C1QA, galectin-1, C/EBP- $\beta$  and FOXO-3A expression – findings considered as a 'molecular signature' of sarcopenia (Giresi *et al.* 2005). A decline in anabolic hormones and reduction in myonuclei via apoptotic-like mechanisms represent alternative explanations for sarcopenia (Leeuwenburgh 2003).

Different inflammatory responses to equivalent exercise also occur in older versus younger muscle. Following 45-min downhill running at 75%  $\text{VO}_{2\text{max}}$  muscle interleukin (IL)-6 and transforming growth factor (TGF)- $\beta$ 1 concentrations did not increase in older ( $n=15$ ) as much as in younger subjects ( $n=15$ ; Carlson *et al.* 1999, Hamada *et al.* 2005, Carlson *et al.* 1999). These differences could reflect alterations in the adaptive cytokine response within the muscle – increasingly evidence points to a role for locally generated IL-6 in assisting with satellite cell proliferation during muscle regeneration following injury (Toumi *et al.* 2006). Decline in anabolic hormones and reduction in available myonuclei via inflammatory or apoptotic-like mechanisms represent alternative explanations for sarcopenia (Leeuwenburgh 2003).

## Muscle atrophy in disuse, denervation and medical disorders

Muscle atrophies with disuse, ageing, denervation, immobilisation, sepsis, neoplasia and chronic disorders, such as HIV/AIDS and rheumatological/multisystem disease. In addition, muscle atrophy is found in states of excess or deficiency of endocrine factors especially glucocorticoids and thyroid hormone. Muscle loss associated with glucocorticoid exposure is augmented by triiodothyronine, a response attenuated by insulin and IGF-I (Sacheck *et al.* 2004). Molecular pathways involved in this process centre on the phosphoinositide 3-kinase–Akt pathway, the potent IGF-I/insulin anabolic pathway and the newly identified transcription factor forked box O class or ‘Foxo’ family (Hoffman & Nader 2004). A further molecule, proteolysis-inducing factor, is being actively investigated (Wyke & Tisdale 2005).

Tumour necrosis factor (TNF)- $\alpha$  alone can induce muscle cell atrophy *in vitro*, reversed by anti-TNF agents possibly via nuclear factor kappa B (NF- $\kappa$ B; Li & Reid 2000). An interaction between TNF- $\alpha$  and IGF-I has been found to be therapeutically relevant where anti-TNF treatment was being provided to patients with rheumatoid arthritis, improving glucocorticoid-associated IGF-I resistance (Sarzi-Puttini *et al.* 2006).

Denervation of muscle (often with associated aberrant reinnervation) is regarded as a potent trigger to sarcopenia – both associated with ageing and neuromuscular disorders – and has recently been examined at the molecular level (Raffaello *et al.* 2006). The use of IGF-I electroporation, recombinant human IGF-I and the peptide representing mechano-growth factor (MGF) have all been considered for ameliorating such neuromuscular conditions, including muscular dystrophy and motor neuron disease (Lynch 2004).

In terms of HIV disease, muscle atrophy occurs both in untreated disease and in association with drug-induced lipodystrophy, endocrine deficiencies, altered protein intake and a cytokine-mediated catabolic state (Yarasheski *et al.* 2005). Using anti-TNF antibodies or receptor blockers represent a therapeutic avenue (Calabrese *et al.* 2004). Several studies have demonstrated a response to androgen therapy in such patients – studied most extensively not only in men (Bhasin *et al.* 2000, Grinspoon *et al.* 2000, Fairfield *et al.* 2001), but also in women (Dolan *et al.* 2004).

Attempts at reversing muscle atrophy in cardiac failure and chronic obstructive airways disease using anabolic therapies have confirmed the presence of hypogonadism, but no significant correlation with respiratory capacity (Laghi *et al.* 2005). In contrast, using a crossover design, short-term testosterone administration improved subjective and objective measures of exercise tolerance and metabolic factors in ten men with angina (Malkin *et al.* 2004). Muscle atrophy secondary to systemic disorders such as cardiac failure, has no unifying aetiology, although a cytokine-mediated process associated with TNF- $\alpha$ , altered nitric oxide synthase expression and neuroendocrine alterations constitute plausible mechanisms (Strassburg *et al.* 2005).

Activation of the renin–angiotensin–aldosterone system, specifically angiotensin-II may also be involved in clinically relevant atrophy. Angiotensin-II infused into rats generates an atrophic response, apparently mediated via skeletal muscle IGF-I reduction; the Akt/mammalian Target of Rapamycin (mTOR)/p70S6K pathway may represent one target (Dalla *et al.* 2001). These changes are reversed upon transgenic IGF-I overexpression (Song *et al.* 2005). Prospective clinical trials examining the effects of manipulations of the renin–angiotensin system, on skeletal muscle in such patients are awaited.

## Specific endocrine systems

### Calcium and vitamin D

Calcium, vitamin D and phosphate levels all impact on muscle function most notably in deficiency states such as the myopathy seen in osteomalacia. This has been confirmed as a histological atrophy of muscle, predominantly type II fibres and is exacerbated by ageing (Janssen *et al.* 2002). Polymorphisms of the vitamin D receptor and vitamin D knockout models have a significant muscle phenotype (Demay 2003). Vitamin D null mice have smaller muscle fibres and raised levels of MRFs. These changes are reversed by treatment with vitamin D (Endo *et al.* 2003). Vitamin D-receptor polymorphisms associated with body composition and muscle strength have been reported in men and women. One study assessing older men showed variation in the vitamin D-receptor FokI polymorphism (Roth *et al.* 2004), with a different polymorphism linked with muscle strength in a further study of older women (Geusens *et al.* 1997). In a longitudinal study looking at sarcopenia in older men and women, low vitamin D was linked with increased risk (by approximately  $\times 2$ ) of reduced muscle mass and strength. A similar relationship was found with raised parathyroid hormone levels (Visser *et al.* 2003). In functional terms, several trials have examined the relationship between vitamin D status and falls; these have been recently reviewed (Mosekilde 2005).

### Glucocorticoids

Glucocorticoid-associated atrophy appears to be specific for type II or phasic muscle fibres. In a study of controlled hypercortisolaemia in healthy men (Ferrando *et al.* 1999), experimental inactivity increased the catabolic effect of glucocorticoids, suggesting the absence of mechanical signals potentiates the glucocorticoid effect. The mechanism of glucocorticoid-induced atrophy may involve upregulation of myostatin and glutamine synthetase, the latter via glucocorticoid receptor interaction with the glutamine synthetase promoter (Carballo-Jane *et al.* 2004). Glucocorticoids inhibit the physiological secretion of growth hormone (GH) and appear to reduce IGF-I activity at target organs – alterations of steroid-induced glutamine synthetase represent a potential mechanism of action, and dose-dependent inhibition of glutamine synthetase was seen using IGF-I in rat L6 cells (Sacheck *et al.* 2004).

### Growth hormone

GH has a variety of anabolic effects and has been shown to increase lean body mass, bone density and reduce fat mass in children, young adult or older adult with GH deficiency (Carroll *et al.* 2004) and those with Turner's syndrome (Carel 2005). GH replacement also improves muscle mass in adult GH deficiency, without significant impact on muscle strength (Hoffman *et al.* 2004). With ageing, GH secretion and systemic IGF-I decrease and GH administration to elderly patients has resulted in changes in body composition seen in younger GH-deficient subjects (Borst 2004). Recombinant GH, when administered to young hypogonadal (Hayes *et al.* 2001) and ageing men (Brill *et al.* 2002), can increase IGF-I mRNA in muscle. In studies combining GH and exercise on muscle strength, a trend towards an increase in fat free mass and myosin heavy chain (MHC) isoforms has been demonstrated (Taaffe *et al.* 1996, Lange *et al.* 2002). Recent work focussing on the molecular adaptations to a 12-week training regime in older men found that GH and exercise together increased the IGF-I isoform MGF gene expression more than each individually (Hameed *et al.* 2004). The mechanisms of GH action and intracellular signalling are not fully understood. Evidence using a myoblast cell line has shown that exogenous GH appears to induce GH-receptor activation, IGF-I mRNA production, phosphorylation of JAK2, Stat5a, 5b and increases in suppressor of cytokine signalling-2 (SOCS-2; Sadowski *et al.* 2001, Frost *et al.* 2002).

Increasing evidence suggests that SOCS-2 is a key negative modulator of GH action (Leroith & Nissley 2005). There are eight SOCS proteins with conserved structural and functional domains (Greenhalgh & Alexander 2004). They appear to show differential responses to systemic insults (Johnson *et al.* 2001). SOCS-2 may have a biphasic concentration-dependent effect (Favre *et al.* 1999, Johnson *et al.* 2001), binding to the Tyr<sup>595</sup> on the GH receptor with low levels inhibiting and higher concentrations enhancing GH effects. The SOCS-2<sup>-/-</sup> knockout mouse shows gigantism and deregulated growth. The mice demonstrate organomegaly, exuberant skeletal development and increased lean mass. The phenotype appears to be different to transgenic models of either GH or IGF-I excess, yet showing features of each (Metcalf *et al.* 2000, Johnson *et al.* 2001). However, a transgenic mouse overexpressing SOCS-2 was paradoxically, also moderately larger than average (Metcalf *et al.* 2000, Johnson *et al.* 2001, Greenhalgh *et al.* 2002).

In a combined GH and SOCS-2 knockout system (combining SOCS-2<sup>-/-</sup> knockout mice with *lit/lit* mice exhibiting a mutation in the GH-releasing hormone receptor), excessive growth only occurred upon GH administration (Greenhalgh *et al.* 2005). This implies a direct role for SOCS in early GH signalling. The SOCS-2 system may be a significant target for future research.

### Insulin-like growth factors

There are two insulin-like growth factors, IGF-I and IGF-II. IGF-I is the only factor known to accelerate both satellite cell proliferation and differentiation and mice transgenically overexpressing IGF-I have significantly higher muscle mass than controls (Hayashi *et al.* 2004, Shavlakadze *et al.* 2005), whilst IGF-II appears to have an important developmental role and has higher specificity for the differentiating capacity of muscle (Florini *et al.* 1996).

IGF-I and IGF-II are both expressed during muscle regeneration and bind IGF-binding proteins (IGFBPs; Hayashi *et al.* 2004, Shavlakadze *et al.* 2005). A recent report has distinguished a mechanism for IGF-II in myogenesis from the classical IGF-I-mediated pathway – when fibroblast growth factor (FGF)-6<sup>-/-</sup> mice were given a regenerative stimulus to their muscle IGF-II and IGF-IIR were specifically upregulated (Armand *et al.* 2004).

In muscle, circulating IGF-I, paracrine generation of local IGF isoforms and IGFBPs may all play a role in myofibre function. It is presently unclear if each IGFBP performs an entirely unique role in skeletal muscle fibres, although several IGFBPs have been linked to time, load and age-dependent differences (Foulstone *et al.* 2003, Spangenburg *et al.* 2003). Evidence is accumulating for IGFBP-5 as a specific activator of myogenesis with independent activity at the muscle cell membrane (Cobb *et al.* 2004).

### IGF-I isoforms and their distribution in muscle

Variation in IGF-I gene expression is based on site-specific alternative splicing of the gene. The gene has six exons, with promoter regions in exons 1 and 2. The mature peptide is found in exons 3 and 4. Transcripts containing exons 1, 3, 4 and 5 or 1, 3, 4 and 6 are called class I; those including 2, 3, 4 and 6 or 2, 3, 4 and 5 are termed as class II. Details of splice variants has recently been identified (Goldspink & Yang 2004), one of linked with mechanical stimuli has been named MGF. Known rat and human isoforms can be summarised as follows (Table 1 and Fig. 2).

The MGF isoform results from a novel splice acceptor site in the intron between exons 5 and 6 altering the structure of the C-terminus. Hameed *et al.* (2003) looked specifically at the differences in gene expression between young and older men following an intense bout of exercise. IGF-IEa was unchanged in both groups and MGF increased specifically in the young men (Fig. 3).

One animal study suggested that mechanically induced MGF mRNA upregulation may decline with age (Owino *et al.* 2001). As yet, there is no unifying explanation for this, but altered mechano-transduction (i.e. cytoskeletal sensitivity to mechanical stimulation) is thought to play a role (Rennie & Wackerhage 2003). The results of key elements of experiments aimed at identifying the role of MGF are shown in Table 2.

**Table 1** Outline of alternative insulin-like growth factor-I (IGF-I) gene splicing in rats and humans

Rats	Humans
IGF-IEa (systemic)	IGF-IEa (systemic)
IGF-IEb (52 bp insert); MGF; comparative to human IGF-IEc	IGF-IEb function unknown IGF-IEc (49 bp insert); MGF

MGF, mechano growth factor.

## Androgens

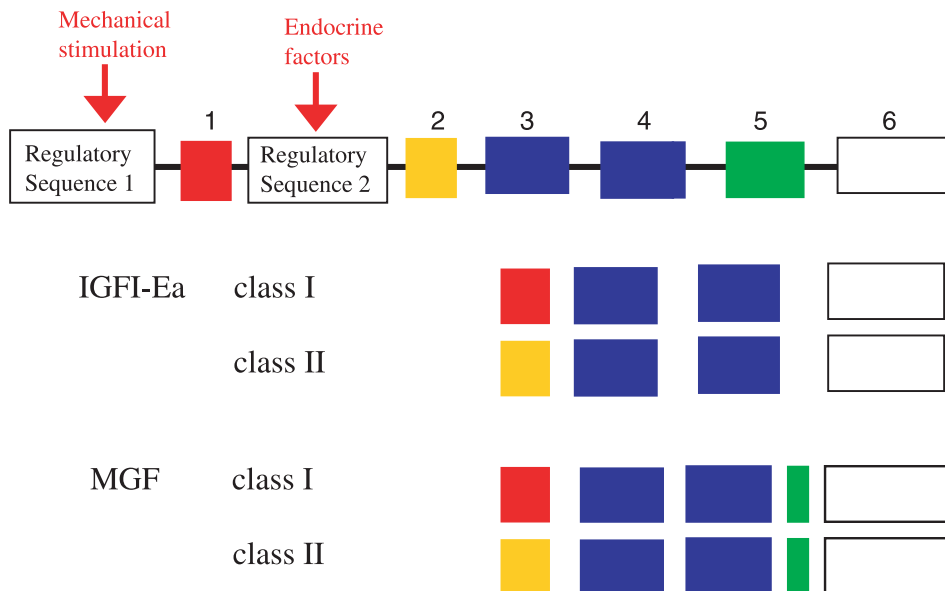
### Mechanisms of androgen action

The key cellular vector for androgen-associated muscle hypertrophy appears to be the satellite cell (Chen *et al.* 2005). Immunohistochemical examination has demonstrated the expression of androgen receptors (ARs) in CD34+ cells, fibroblasts, smooth muscle cells and satellite cells (Sinha-Hikim *et al.* 2004). Murine pluripotent C3H10T1/2 cells have been used to demonstrate increased MyoD+ and MHC+ myotubes following androgen administration, with dynamic effects of an AR antagonist (Artaza *et al.* 2005). The role of the androgen receptor has been studied using C2C12 myoblast satellite cells transfected with a flagged AR–accelerated myoblast cell differentiation was seen via enhanced myogenin expression (Lee 2002). Using C2C12 myoblasts transfected with wild-type or mutant ARs, testosterone induced a variable rate of cell differentiation or proliferation (Benjamin *et al.* 2004), androgen action using a gonadotrophin-releasing hormone agonist in young men resulted in a dose–response increase in satellite cell number in muscle biopsy samples (Sinha-Hikim *et al.* 2003).

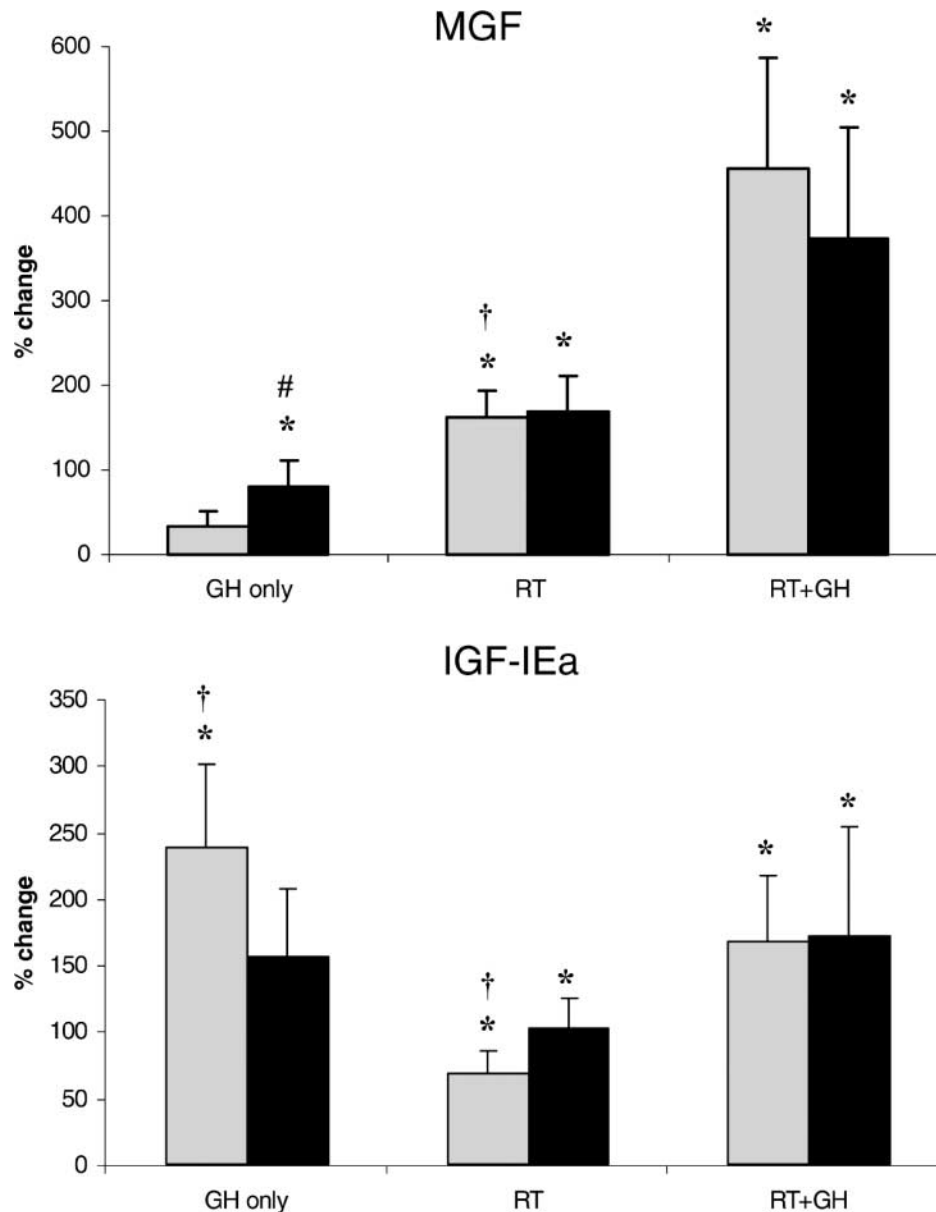
Functionally important AR polymorphisms, including effects on body composition, have been recently associated with the

cytosine adenine guanine (CAG) triplet repeat motif (Walsh *et al.* 2005). A pathological lengthening of CAG repeat is seen in Kennedy disease, an X-linked spinobulbar muscular atrophy (Merry 2005). When a cohort of patients with Kennedy disease were analysed in detail, a correlation was found between CAG repeat length and clinical manifestations with >60% subjects showing evidence of androgen insensitivity (Dejager *et al.* 2002).

There is increasing evidence of androgen action in muscle interacting with IGF-I. In rat diaphragmatic muscle, a dose-dependent increase in IGF-I mRNA expression occurred following exposure to androgen (Lewis *et al.* 2002). When harvested bovine male satellite cells were treated with various androgen concentrations (using trenbolone), a dose-dependent increase in IGF-I mRNA expression occurred (Kamanga-Sollo *et al.* 2004). As yet no data have been generated demonstrating a distinction in dynamics of specific IGF-I isoforms upon androgen administration. Androgenic activity may be directly linked to the IGF-I signalling system with p70s6kinase, found downstream of the Akt/mTOR pathway, a candidate for early evidence is emerging (Xu *et al.* 2004). A proportion of the anabolic effects of androgens may also be anti-catabolic via an anti-glucocorticoid action (Danhaive & Rousseau 1988, Zhao *et al.* 2004).



**Figure 2** Diagram showing structure of IGF-I splice variants.



**Figure 3** Changes in MGF and IGF-IEa levels in elderly men in three groups exposed to growth hormone (GH), resistance training (RH) or both (GH + RT). Values are expressed as percentage change from baseline at 5 (grey bars) or 12 (black bars) weeks. Bars and error bars represent mean values and s.e.m respectively. \*Significant difference from baseline  $n$ , #significant difference from 5 weeks ( $P < 0.05$ ); †significant difference in percentage change between isoforms ( $P < 0.05$ ). Hameed M, Lange KH, Andersen JL, Schjerling P, Kjaer M, Harridge SD & Goldspink G 2004 The effect of recombinant human growth hormone and resistance training on IGF-I mRNA expression in the muscles of elderly men. *Journal of Physiology* 555 231–240. Reprinted with permission from Blackwell Publishing.

#### Clinical studies demonstrating the interaction of androgens and muscle

Androgens are associated with muscle size and strength, with a complex association between androgen levels and mechanical performance. In younger subjects, both androgens and exercise have been shown to increase muscle size and strength alone and

in combination (Bhasin *et al.* 1996). More recently, effects have also been observed in older subjects, albeit of reduced magnitude (Bhasin *et al.* 2001, 2005). In a study of ten older men sequentially exposed to testosterone; GH or both, muscle IGF-I gene expression rose 1.9-fold in the GH group and by 2.3-fold in the testosterone + GH groups (Brill *et al.* 2002).

**Table 2** Summary of data on the effects of the IGF-I splice variant known as MGF

<i>In vitro</i>	Animal	Human
C2C12 cells overexpressing MGF associated with myoblast proliferation; inhibition of terminal differentiation.	Increased expression of MGF upon isolated limb mechanical loading, attenuated in older animals.	Expression of MGF in muscle increased upon exercise and splicing towards MGF expression increasing on exposure to GH with a more significant response seen in younger subjects versus older subjects.
Upregulation of MGF on GH administration and/or mechanical stimulus to muscle cells.	Injection of MGF into mouse muscle appears to improve muscle fibre size and strength.	

More recently, larger ( $n=80$ ) placebo-controlled randomised trial of GH versus testosterone or both, a statistically significant effect on muscle size and body composition was only found in the combination group (Giannoulis *et al.* 2006). However, only a fixed dose of testosterone was used. Therefore, future research may attempt to explore the mechanism of an androgen-mediated effect on the GH/IGF-I system.

#### Synthetic androgen modulators

Exogenous anabolic androgenic steroids such as oxandrolone have demonstrable efficacy, and are associated with increased AR mRNA and muscle protein synthesis (Sheffield-Moore *et al.* 1999). This effect was recently documented at the level of gene expression using microarray expression profiling where tetrahydrogestinone, a notorious synthetic anabolic androgen, was found to have a remarkably similar profile to dihydrotestosterone (Labrie *et al.* 2005).

A promising new androgen therapy 7 $\alpha$ -methyl-19-nortestosterone abbreviated as 'MENT' (also called trestolone) offers a number of clinical advantages when compared with conventional androgens. It can be delivered by several routes and is aromatisable, but not a substrate for 5 $\alpha$ -reductase. A study using MENT in orchidectomised rats showed anabolic effects on muscle and bone (Venken *et al.* 2005). Although the compound showed a dose-related action on the prostate, the higher doses tested caused both muscle gain and prostate hypertrophy the low to mid doses had divergent effects, with a smaller effect on the prostate. Therefore, dose-finding prospective randomised placebo-controlled clinical studies will be crucial (Anderson *et al.* 2003).

A further therapeutic mechanism in development is the use of selective AR modulators (SARMs), an exciting development aiming to provide anabolic effects without widespread adverse effects (Negro-Vilar 1999). Presently, the mechanism for tissue selectivity is still being explored (Gronemeyer *et al.* 2004). Initial development was derived from modification of the AR antagonists bicalutamide and flutamide. A study using orchidectomised rats using the 'S-4' compound demonstrated full agonist activity in muscle, but partial agonist action in the prostate providing improvements in muscle mass, strength and bone density without adverse prostatic effects (Gao *et al.* 2005). As yet, there are no convincing human data using

orally available SARMs and examining muscle-related outcomes, although phase I/II trials are under way.

#### Myostatin

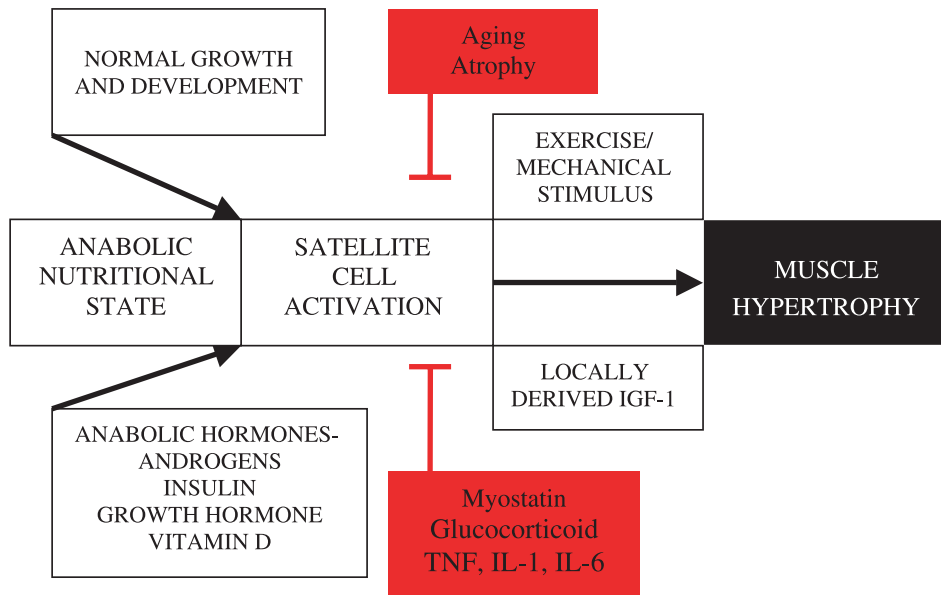
Myostatin was first discovered by McPherron *et al.* (1997) who demonstrated that a phenotype of exaggerated muscle hypertrophy correlated with mutations in the myostatin gene. Such knockout mutations of myostatin in animals (Grobet *et al.* 1997) – causing the so-called 'double-muscling' phenotype – and in one human child (Schuelke *et al.* 2004) have been described. Myostatin knockout mice show increased satellite cell proliferation compared with wild-type controls (Wagner *et al.* 2005), physiologically, it thus appears to be a gatekeeper maintaining satellite cells in reversible quiescence (Amthor *et al.* 2006).

The gene is highly conserved across species and polymorphisms of the myostatin gene are correlated with measures of muscle mass, strength and potentially athletic performance (Seibert *et al.* 2001). Also known as growth and differentiation factor 8, it is a member of the TGF superfamily. Myostatin acts through activin type I receptors, ActR2A and ActR2B. So far evidence suggests important binding properties to the propeptide itself and the follistatin-related gene protein (Hill *et al.* 2002). Follistatin appears to be a complementary antagonist to myostatin (Amthor *et al.* 2004). Myostatin acts as an inhibitor of muscle growth and promotes adipogenesis (Artaza *et al.* 2005). Myostatin signalling involves interaction with the MRFs, inhibiting the synthesis and activity of MyoD (Amthor *et al.* 2004, Guttridge 2004). At the cellular level, myostatin affects cell cycling by affecting the entry of satellite cells into S-phase (McCroskey *et al.* 2003, Amthor *et al.* 2004).

#### Endocrine targets of myostatin action

Myostatin appears to be sensitive to the anabolic actions of GH. Using the C2C12 myotube model, a dose-dependent reduction in myostatin expression was observed on exposure to GH, which was reversed by the GH antagonist pegvisomant (Liu *et al.* 2003).

In contrast, dexamethasone has been shown to increase myostatin expression that could be antagonised by RU-486



**Figure 4** Diagram designed to represent positive and negative factors leading to muscle hypertrophy via activation of satellite cells.

(Ma *et al.* 2001). The human myostatin gene promoter has been found to contain a number of potential glucocorticoid-response elements (Ma *et al.* 2001). Myostatin is also exercise responsive. In rats performing an exercise regime comprising 5 days of swimming, myostatin mRNA was significantly reduced in the exercise group compared with controls (Matsakas *et al.* 2005). In contrast, a clinical study using a 12-week resistance training protocol in young men found concomitant increases in glucocorticoid receptor and myostatin mRNA expressions (Willoughby 2004). Another report using a human training schedule demonstrated myostatin reduction (mean 20%) in the serum following heavy exercise (Lin *et al.* 2003, Walker *et al.* 2004). The explanation for these conflicting data is uncertain.

A variety of conditions causing muscle loss associated with observable increase in myostatin levels, including sarcopenia of ageing, HIV disease, disease disuse atrophy, glucocorticoid use and microgravity during spaced flight (Gonzalez-Cadavid *et al.* 1998, Baumann *et al.* 2003). In addition, male transgenic mice overexpressing myostatin had reduced muscle and higher fat mass than females (Reisz-Porszasz *et al.* 2003). Gender-specific evidence such as this leads to the suggestion that androgens could have a role in the mechanism.

#### Clinical trials involving myostatin inhibition

A recombinant human antibody to myostatin (MYO-029) is being utilised with the aim of improving states of muscle wasting in neuromuscular disease in early phase trials. A second therapeutic approach now in development is a soluble activin type IIB receptor (ACVR2B/Fc) that binds to myostatin effectively causing reduced myostatin availability

(Lee *et al.* 2005). These data are interesting in that normal animals were used, making this a transferable paradigm for sarcopenia research.

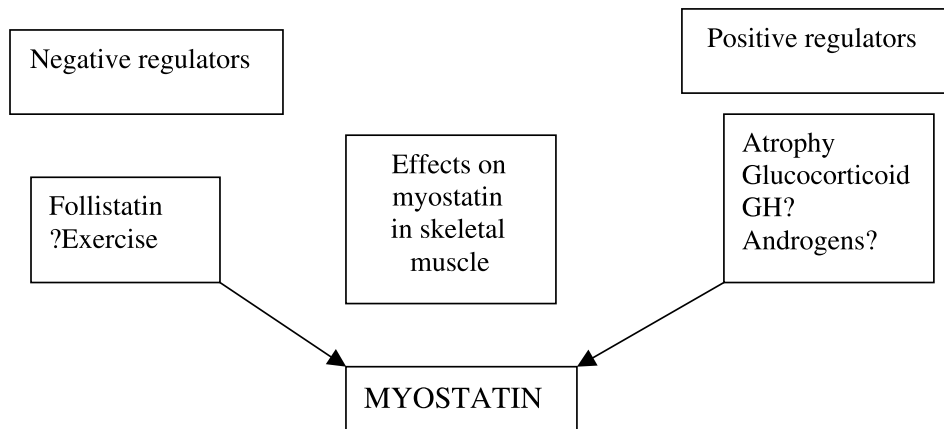
Figure 4 shows the differing endocrine influences on satellite cell function; Fig. 5 specifies effects on fibre types; Fig. 6 summarises endocrine factors influencing muscle-derived myostatin specifically.

#### Using muscle in hormonal gene transfer

Muscle mass can also be influenced using plasmid gene transfer approaches to generate a variety of target endocrine

	Type I	Type II
Androgens	++	++
Myostatin	–	–
Glucocorticoid		–
Vitamin D deficiency		–
T4 deficiency		–

**Figure 5** Recognised effects of endocrine factors on dynamics of type I (slow) and type II (fast) muscle fibres; + denotes anabolic effect; – denotes negative regulatory or catabolic effect.



**Figure 6** Outline of endocrine interactions affecting dynamics of myostatin.

molecules (Goldspink 2003, Reisz-Porszasz *et al.* 2003). MacColl *et al.* (2000) successfully employed this approach using GH as the target. The C2C12 model of murine muscle cells was used and GH secretion was measurable under several transfection conditions, including various stages of cellular differentiation into syncytial myotubes. This and other approaches may soon have a significant impact on the field of endocrine biology and a possible new source for anabolic hormones in deficiency states.

## Conclusion

The range of endocrine factors operating upon skeletal muscle as a target organ is extensive. Recent advances have occurred in our understanding of IGF-I splice variants, the targets of androgen action and the role of myostatin. Interaction between growth factors and mechanical stimulation is of significant importance, although the underlying processes involved remain unresolved. In addition, further intense research into the biology of satellite cells will enable the harnessing of their stem cell properties under optimal endocrine conditions.

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