REVIEW

Androgen regulation of satellite cell function

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Abstract

Androgen treatment can enhance the size and strength of muscle. However, the mechanisms of androgen action in skeletal muscle are poorly understood. This review discusses potential mechanisms by which androgens regulate satellite cell activation and function. Studies have demonstrated that androgen administration increases satellite cell numbers in animals and humans in a dose–dependent manner. Moreover, androgens increase androgen receptor levels in satellite cells. *In vitro*, the results are contradictory as to whether androgens regulate satellite cell proliferation or differentiation. IGF-I is one major target of androgen action in satellite cells. In addition, the possibility of non-genomic actions of androgens on satellite cells is discussed. In summary, this review focuses on exploring potential mechanisms through which androgens regulate satellite cells, by analyzing developments from research in this area.

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Introduction

With increasing age, skeletal muscle mass is lost, and aging atropy is accompanied by a reduction in muscle strength that contributes to frailty and falls (Cornelison & Wold 1997). The frailty of old age has emerged as an important public health problem because it impacts mobility and quality of life and substantially increases the use of health care resources (Bross *et al.* 1999). Because of the age-associated decrease in testosterone serum levels in men (Snyder 2001, Yialamas & Hayes 2003), recent years have witnessed a growing interest in the use of androgenic interventions for augmenting muscle mass and function in older men. Therefore, there is a great need for research on the mechanisms of androgen action in skeletal muscle.

The anabolic actions of androgens can enhance muscle strength and increase muscle size clinically (Brodsky *et al.* 1996, Bhasin *et al.* 1997, 2001*a,b*, Mauras *et al.* 1998, Sinha-Hikim *et al.* 2002). *In vivo*, androgens can increase skeletal muscle mass (Nnodim 1999*b*). However the biological mechanisms of androgen action in skeletal muscle are poorly understood.

Myoblasts are committed myogenic cells that are the main source of muscle growth and regeneration. When myoblasts are quiescent, they are termed satellite cells. After satellite cells are activated to become myoblasts, they enter the proliferation stage and differentiate into myotubes, which finally fuse with existing myofibers that give rise to muscle. Therefore, this review will focus on the relationship between androgens and satellite cells in order to explore the underlying mechanisms of the stimulatory role of androgens in skeletal muscle.

Androgens and the androgen receptor

Androgens are crucial for the establishment and maintenance of reproductive organs in men. In addition, androgens have anabolic actions in other tissues including muscle and bone (Yin *et al.* 2003). Testosterone is the principal circulating androgen, secreted by testicular Leydig cells under luteinizing hormone stimulation (Mooradian *et al.* 1987). In reproductive tissues, testosterone is converted to dihydrotestosterone (DHT) via 5α-reductase. Testosterone is the main androgen in skeletal muscle because of low levels of 5α-reductase in muscle (Bhasin *et al.* 2003*a*).

Androgens are ligands of the androgen receptor (AR). Androgens bind to the AR to form complexes that bind promoter or enhancer elements of target genes to regulate their transcription. The AR, like other steroid receptors, is composed of relatively discrete functional domains (Fig. 1): (i) a hypervariable N-terminal domain which regulates transcriptional activity, (ii) a central highly conserved DNA-binding domain, (iii) a short hinge region, and (iv) a large C-terminal, ligand-binding domain (Evans 1988).

The AR modulates target gene transcription via recognition and binding of the androgen response elements

Androgen Receptor Gene

N-terminal   DNA-binding   Hinge   Ligand-binding

Androgen Receptor Protein
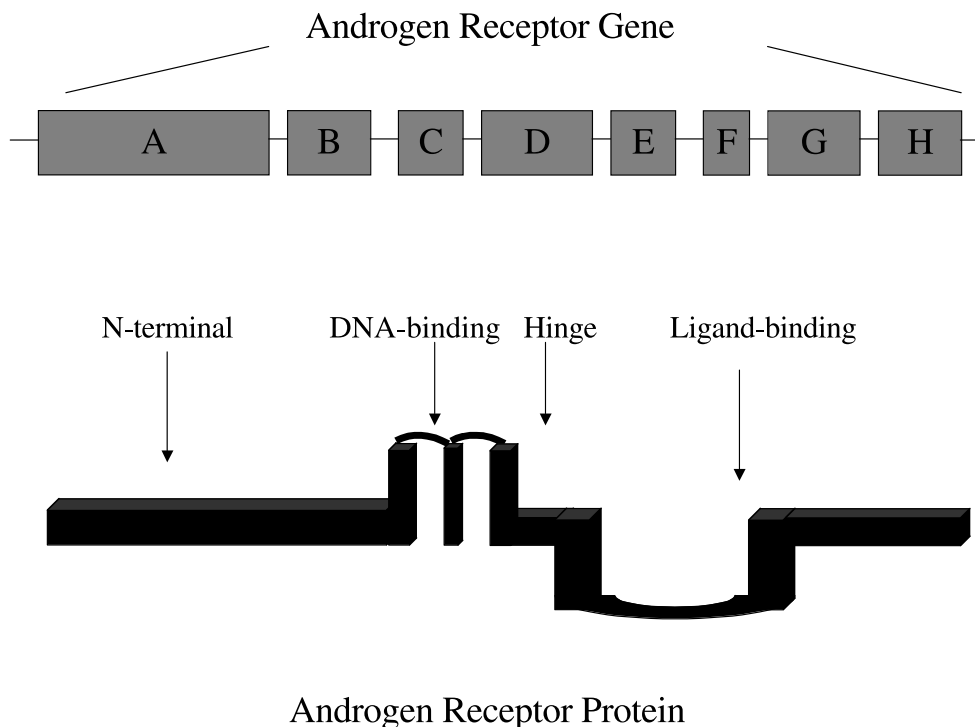
Figure 1 Diagram of androgen receptor gene and functional domains. Exon A encodes the N-terminal transactivation domain, and exons B and C encode the DNA-binding domain. Exon D encodes the hinge region, and the 3′ end of exon D and exons E-H encode the ligand binding domain.

(AREs) (Freedman 1992). The AR functions only after binding its hormonal ligands (androgens), which induces transformation of the receptor and results in dimerization, nuclear localization and DNA binding. The AR binds to its AREs as a homodimer, with each monomer contacting one half-site of the palindromic responsive element (Wong et al. 1993). The classical function of the AR is to modulate target gene expression.

Satellite cells

The satellite cell, defined on the basis of location, is a mononucleated cell that lies under or embedded in the basal lamina of the myofiber, which demonstrates close relationship with the mature myofiber (Campion 1984, Grounds et al. 2002). During development and regeneration, quiescent satellite cells are activated and start proliferating, at which stage they are often referred to as myogenic precursor cells or myoblasts (Charge & Rudnicki 2004). Myoblasts further differentiate into post–mitotic myotubes that eventually fuse with myo–fibers (Fig. 2). The primary myogenic regulatory factor (MRF), MyoD, is required for the determination of myoblasts, whereas the secondary MRFs, myogenin and MRF4, function to regulate terminal differentiation

MRFs

Satellite cells   Myf5

Activation →   MyoD

Myoblasts   Proliferation

Differentiation →   Myogenin
MRF4
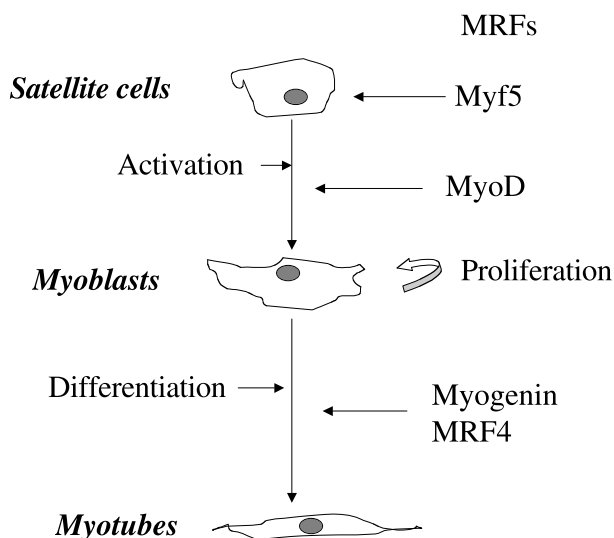
Myotubes

Figure 2 Relationship between satellite cells, myoblasts and myotubes. Satellite cells are activated and become myoblasts after expressing MyoD. Myoblasts are the proliferative cells of skeletal muscle, which exit from proliferation and differentiate into myotubes associated with expression of myogenin and MRF4. MRFs, myogenic regulatory factors.

(Seale & Rudnicki 2000). In adult skeletal muscle, new myonuclei are only required for growth and repair. Accordingly, the satellite cells are normally quiescent and are activated only in response to stimuli elicited by damage or work load (Seale & Rudnicki 2000).

Early studies suggested that the somatic mesoderm gives rise to satellite cells (Armand *et al*. 1983). The study carried out by De Angelis *et al*. (1999) showed that a subset of satellite cells also comes from the dorsal aorta that is derived from the paraxial mesoderm. The coexpression of a number of endothelial and myogenic markers in cells derived from the dorsal aorta suggests that these myogenic cells may be derived from true endothelial cells or from a common precursor.

Quantification and distribution

Satellite cell number is not constant throughout life and is dependent on age and muscle fiber type. Satellite cells are most abundant during early development when they contribute to muscle growth, and decline in number thereafter. Studies in the rat showed that the absolute number of satellite cells was reduced by 22% in older animals compared with their younger counterparts (Nnodim 2000). In human, the number of satellite cells also decreases with increasing age (Kadi *et al*. 2004). Moreover, individual muscles vary in satellite cell population dynamics. In the rat extensor digitorum longus, which is a fast twitch muscle, the number and rate of proliferation of myonuclei is lower than in the slow twitch soleus muscle (Campion 1984). This correlates with the finding of a relatively smaller satellite cell population and a more rapid decline with age in the satellite cell number in the extensor digitorum longus muscle than in the soleus muscle (Campion 1984). The differences in satellite cell distribution between muscle groups is a result of the heterogeneity in satellite cell content between muscle fiber types (Hawke & Geary 2001).

Physiology

There is functional evidence that satellite cells are a heterogeneous population. *In vitro*, clonal studies have indicated that satellite cells isolated from adult skeletal muscle are heterogeneous in terms of cell size and clonogenic potential (Zammit & Beauchamp 2001). Different subpopulations of satellite cells can also be defined by their behavior and markers. Studies on skeletal muscle of rat found that 80% of satellite cells divide rapidly and are responsible chiefly for providing myonuclei to growing fibers. The remaining 20% of the cells, regarded as reserve cells, divide more slowly due to most of the cells entering the G_0 phase (Schultz 1996). The expression of the markers CD34, Myf5 and M-cadherin define 80% of satellite cells, while the other 20% of cells do not express these genes (Beauchamp *et al*. 2000), which is consistent with the division behavior of satellite cells. Taken together, these data show that there are distinct subpopulations in satellite cells.

It is likely that the satellite cell pool would become rapidly exhausted without renewal (Zammit & Beauchamp 2001). Therefore, satellite cells have a capacity for self-maintenance (Bischoff 1994). However, the mechanism by which satellite cells undergo self-renewal in the adult is not well understood (Schultz 1996). Asymmetric division of satellite cells, dedifferentiation of committed myogenic cells and contribution from stem cells may be the underlying mechanisms (Schultz 1996, Gussoni *et al*. 1999, Beauchamp *et al*. 2000, Asakura *et al*. 2002, Zammit *et al*. 2004).

Striking differences can be observed in skeletal muscle between the adult and the aged in terms of muscle size and strength. One mechanism could be the decrease in the number of satellite cells with aging (Nnodim 2000). Another explanation is that the loss of muscle mass that occurs with aging is likely to reflect changes to the *in vivo* microenvironment required for the efficient activation and differentiation of satellite cells (Renault *et al*. 2000, Seale *et al*. 2001, Charge *et al*. 2002).

Androgen regulation of satellite cell function

Androgens have long been regarded as having positive effects on muscle size and strength. Clinically, androgens have been used to increase muscle bulk in hypogonadal men (Bhasin *et al*. 1997), HIV-infected men (Bhasin *et al*. 2000) and normal men (Bhasin *et al*. 1996, 2001*b*). In addition, animal studies also show anabolic effects of androgen on muscle size and strength (Antonio *et al*. 1999, Nnodim 1999*a*). Satellite cells are believed to be a direct target of androgens because they express the AR (Doumit *et al*. 1996). However, the underlying mechanisms by which androgen regulates satellite cells are still not clear.

Human research

Effects of androgens on young hypogonadal and healthy men Several studies have evaluated the effects of androgen on muscle in hypogonadal men. Bhasin *et al*. (1997) found arm and leg muscle cross-sectional areas, assessed by magnetic resonance imaging, increased significantly after testosterone treatment. Substantial increases in muscle strength were also noted. Another study demonstrated that muscle mass increased by 20% and accounted for 65% of the increase in fat-free mass when hypogonadal men were treated with testosterone enanthate (Brodsky *et al*. 1996). Testosterone-induced increase in muscle size in eugonadal young men was associated with muscle fiber hypertropy (Bhasin *et al*. 2001*b*). The treatment was associated with a dose-dependent increase in myonuclear

number (Sinha-Hikim *et al.* 2002). Therefore, testosterone increases skeletal muscle size partly by inducing muscle fiber hypertropy.

Effects of androgens on aging men Testosterone may also potentially improve muscle strength and size in older men with low testosterone concentrations. One study (Schroeder *et al.* 2003*b*) revealed that treatment with the androgen, oxymetholone, significantly augmented total lean body mass and maximum voluntary strength in older men. These changes were quantitively related to the dose of oxymetholone. In another study (Schroeder *et al.* 2003*a*), the relative change in muscle cross-sectional area of the total thigh in a group treated with oxandrolone increased significantly compared with placebo treatment. Testosterone supplementation is effective for increases in muscle size and strength in healthy older men with low–normal or mildly decreased testosterone levels (Gruenewald & Matsumoto 2003).

Androgen supplementation in patients with muscle wasting associated with chronic illness Studies on the effects of androgen supplementation in HIV-infected men have been reported (Bhasin *et al.* 2000). Men treated with testosterone experienced significant increases in maximum voluntary muscle strength, associated with an increase in muscle volume (Sattler *et al.* 1999, Bhasin *et al.* 2000). Weight loss in chronic obstructive pulmonary disease (COPD) is associated with skeletal muscle dysfunction and increased mortality (Yeh *et al.* 2002). It has been shown that there is a greater prevalence of hypogonadism in patients with COPD compared with normal men (Debigare *et al.* 2003). Improvements in muscle function and muscle size have been observed in COPD patients treated with nandrolone (Creutzberg & Schols 1999, Creutzberg *et al.* 2003). Androgens have also been used in burns patients. Studies have demonstrated that testosterone administration can restore an important anabolic stimulus to skeletal muscle and ameliorate nitrogen loss, with a significant increase in lean mass in the androgen-treated burns subjects (Ferrando *et al.* 2001, Wolf *et al.* 2003).

Androgens in athletic performance There is agreement that testosterone supplementation increases maximal voluntary strength of muscle (Sattler *et al.* 1999, Bhasin *et al.* 2000). A study comparing weight-lifters with and without a history of androgen administration showed that the sizes of myofibers from athletes in the former group were larger than those of athletes in the latter group (Kadi *et al.* 1999). Therefore, it is not surprising that the abuse of androgenic steroids is most prevalent among power lifters. Testosterone may also enhance recovery from exercise and improve explosive powers (Bhasin *et al.* 2001*a*), which could contribute to the abuse of androgen in sprint and short-distance swimming events.

Collectively, these data from studies on humans unequivocally indicate that androgens can increase muscle size and maximal voluntary strength in humans. However, there is only limited use of androgens in clinical practice.

Androgen receptor in muscle

AR expression has been identified in rat skeletal muscle by androgen binding (Krieg 1976, Michel & Baulieu 1980). *In vitro*, the AR protein has been demonstrated in porcine satellite cells (Doumit *et al.* 1996). Taken together, these data suggest that androgens can act directly through the AR expressed in satellite cells.

Direct regulation of satellite cells by androgens

Increase in satellite cell number Androgen administration can increase satellite cell number in animals and humans. In rats, treatment with testosterone before the onset of puberty induced a marked but transient increase in lavator ani muscle satellite cell number not only in males but also in females (Joubert *et al.* 1994). This cell proliferation was followed by a subsequent increase in the myonuclei number (Joubert & Tobin 1989). Researchers from the same group confirmed in rat levator ani muscle that quiescent satellite cells could be activated and recruited into the cell cycle after testosterone treatment (Joubert & Tobin 1995). One study (Nnodim 2001) compared denervated levator ani muscles of castrated adult male rats with or without testosterone treatment. The satellite cell number was approximately an order of magnitude greater in the testosterone-treated rats than in the untreated rats. Another study revealed that castration reduced satellite cell proliferation in pigs (Mulvaney *et al.* 1988), while castrated pigs treated with testosterone treatment had greater satellite cell proliferation. Taken together these studies show that androgens increase the number of satellite cells.

In athletes, Kadi *et al.* (1999) studied muscle biopsied from weight-lifters with or without recorded use of anabolic steroids. The mean number of nuclei in myofibers was significantly higher in the reported steroid-using athletes than in the nonsteroid-using athletes. There was a nearly fivefold increase in the proportion of fibers with central nuclei in the reported steroid-using athletes in comparison with the other athletes. Because the nuclei within the muscle fibers are postmitotic, new myonuclei must be contributed by the satellite cells (Allen *et al.* 1999, Sinha-Hikim *et al.* 2003). This observation is in accordance with a previous study in rats (Joubert *et al.* 1994) that showed that muscle fiber hypertropy induced by testosterone was characterized by an increase in the number of myonuclei. This increase in myonuclear number was preceded by an increase in satellite cell proliferation (Kadi *et al.* 1999).

Table 1 Summary of *in vitro* studies examining effects of androgens on proliferation and differentiation of satellite cells or myoblasts

	Androgen treatment	Effects on proliferation	Effects on differentiation	Study
Target cells				
Primary myoblasts (rat)	Testosterone	Enhanced proliferation	Not examined	Powers & Florini 1975
Satellite cells (rat)	Trenbolone	No effect	Not examined	Thompson *et al.* 1989
Satellite cells (pig)	Testosterone	No effect	Reduction in differentiation	Doumit *et al.* 1996
C2C12 cells (mouse myoblast cell line)	Testosterone	No effect	Increase in differentiation	Lee 2002

Studies performed by the Bhasin group explored the relationship between satellite cell number, muscle size and androgen treatment in humans (Sinha-Hikim *et al.* 2002, 2003). Testosterone-induced muscle fiber hypertrophy was associated with an increase in myonuclear number, which was greater in men receiving high doses (Sinha-Hikim *et al.* 2003). An increase in satellite cell number was also observed, which was significantly correlated with the change in testosterone concentration. The authors concluded that an increase in satellite cell number and the subsequent fusion of satellite cells with muscle fibers resulted in an increase in myonuclear number and muscle fiber hypertrophy (Sinha-Hikim *et al.* 2003). These data confirm that a testosterone-induced increase in satellite cell number also takes place in humans.

***In vitro* studies** Although studies demonstrate that androgens increase satellite cell number, the mechanisms underlying this effect are not known. It is still not clear at which stage of satellite cell function androgens act, because conflicting results have been reported (Table 1). Powers & Florini (1975) reported a direct effect of testosterone on proliferation of rat primary myoblasts in culture. The thymidine labeling index, a measurement of cell proliferation, was enhanced by androgens to a modest degree. However, another study found no direct actions of androgens on rat satellite cell proliferation *in vitro* (Thompson *et al.* 1989). Similarly, another study using porcine satellite cells treated with different doses of testosterone showed no measurable effect on proliferation (Doumit *et al.* 1996). However, testosterone suppressed myotube formation in a dose-dependent manner, which suggested that increases in the population of proliferative satellite cells *in vivo* may be manifested, at least in part, by a reduction in cell differentiation (Doumit *et al.* 1996). The presence and autoregulation of AR in satellite cells were also observed, indicating that satellite cells are targets for androgen action. A contrasting study (Lee 2002) in the C2C12 mouse myoblast cell line suggested that androgens accelerated skeletal myoblast differentiation associated with up-regulation of myogenin expression. These inconsistent data indicate that further well-controlled *in vitro* studies are warranted.

Increase in AR levels Up-regulation of AR levels is one of the documented responses to androgens in target tissues or organs. Up-regulation of AR levels by androgen treatment in skeletal muscle has been observed in rat and human (Antonio *et al.* 1999, Kadi *et al.* 2000, Lee *et al.* 2003). The auto-regulation of AR levels by androgens may occur through stabilizing existing receptors or by increasing *de novo* receptor synthesis (Kadi *et al.* 2000). Up-regulation of AR levels by androgens could be one mechanism by which androgens have effects on muscle.

The increase in AR levels with androgen treatment has been demonstrated in satellite cells in pig (Doumit *et al.* 1996). This study clearly demonstrated the presence and auto-regulation of AR in satellite cells and also myotubes. Immunoblot analysis revealed that AR expression in satellite cells and myotubes was up-regulated in response to testosterone. Moreover, immunocytochemical staining for AR was more intense in the nuclei of satellite cells and myotubes from androgen-treated cells. Because the AR is located in the myonucleus, the increased nuclear number could potentially give rise to an elevation in the number of androgen binding sites (Kadi *et al.* 1999). Taken together, these data suggest that androgens may have effects on satellite cells through up-regulation of AR levels in satellite cells, which could enhance the sensitivity of satellite cells to androgens.

Indirect regulation of satellite cells by androgens

In addition to direct actions, indirect actions of androgens in muscle growth and regeneration via other signaling pathways have been proposed (Fig. 3).

Insulin–like growth factor–I In recent years, it has become increasingly apparent that the autocrine/paracrine insulin-like growth factor (IGF) system within skeletal muscle plays an important role in myogenesis and maintenance of muscle fiber growth (Lewis *et al.* 2002). IGF-I is ubiquitously expressed in skeletal muscle and appears to be important in both the proliferation and differentiation of skeletal muscle myoblasts (Florini *et al.* 1996, Layne & Farmer 1999). This results in skeletal muscle hypertrophy (Adams 1998).

There are two splice variants of the IGF-I gene expressed in skeletal muscle, mechanogrowth factor (MGF) and IGF-IEa (Goldspink & Harridge 2004). The former is mechanosensitive and autocrine, while the latter is similar to the systemic or liver type of IGF-I.
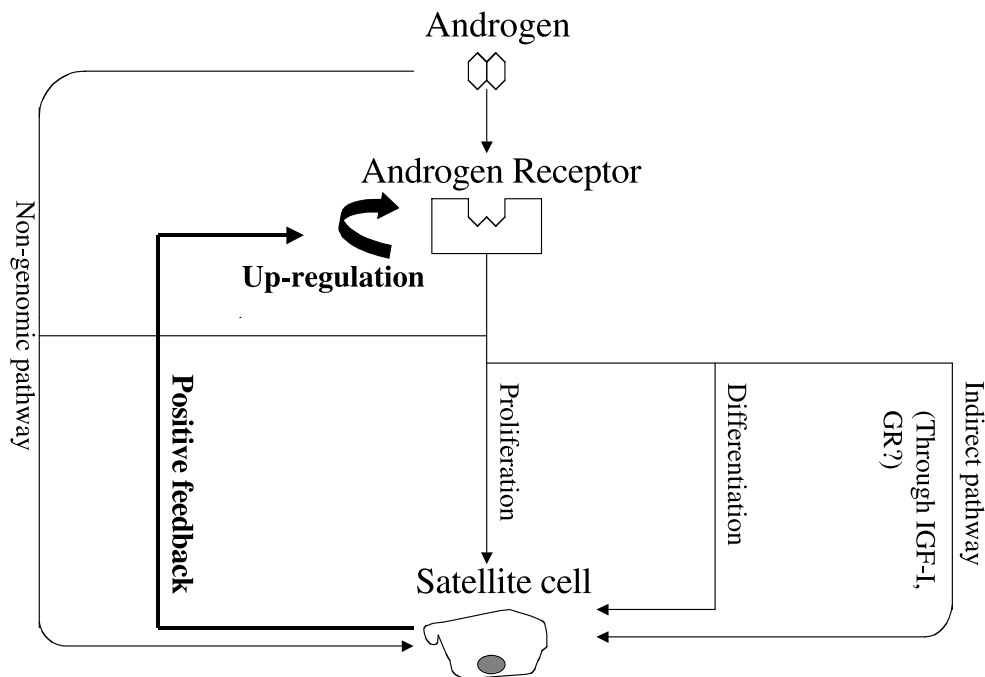
Figure 3 Possible mechanisms by which androgens regulate satellite cells. The first mechanism is the classical AR-mediated pathway, where androgens may exert effects on proliferation or differentiation of satellite cells. Up-regulation of AR levels in satellite cells is the result of positive feedback from this pathway. The alternative pathway could be through non-genomic actions of androgens. The indirect pathways, including those mediated via IGF-I or the GR, may be another mechanism by which androgens act on satellite cells.

MGF differs from IGF–IEa in having a 49–base insert in the last coding exon of the mRNA that results in a reading frameshift and hence a different carboxy peptide sequence. Different IGF–I isoforms have different actions. *In vitro*, C2C12 cells overexpressing MGF caused increased myo-blast proliferation and inhibited terminal differentiation, while C2C12 cells overexpressing IGF–IEa differentiated into myotubes (Yang & Goldspink 2002), with a smaller stimulation of proliferation. Another study indicated that an initial pulse of MGF expression following muscle damage activates satellite cells, and this is followed by late expression of IGF–IEa to maintain protein synthesis to complete muscle repair (Hill *et al.* 2003). In addition, these two isoforms of IGF–I are also differentially regulated in skeletal muscle. One study showed that in C2C12 cells MGF was expressed at a very low level under basal static culture but was markedly up-regulated by mechanical loading. In contrast, IGF–IEa was constitutively expressed in proliferation and differentiated C2C12 cells and was stimulated by a single ramp stretch but was reduced by repeated cycle stretch (Cheema *et al.* 2005).

Recent studies have suggested that the anabolic effects of androgens in muscle may be mediated, in part, by local IGF–I (Lewis *et al.* 2002). In rats, the administration of nandrolone produced significantly higher levels of muscle IGF–I compared with rats without nandrolone treatment

(Lewis *et al.* 2002). Androgen treatment was also found to increase IGF-I mRNA in bovine satellite cells (Kamanga-Sollo *et al.* 2004). In humans, IGF-I protein expression in skeletal muscle increased after testosterone treatment (Ferrando *et al.* 2002). Another study showed mRNA concentrations of IGF-I decreased significantly in men treated for 10 weeks with a gonadotropin–releasing hor-mone (GnRH) analog to make them testosterone deficient (Mauras *et al.* 1998). By contrast, circulating levels of IGF-I remained stable. This suggests that one of the primary anabolic effects of androgens may be their ability to stimulate IGF–I production in skeletal muscle, which is independent of systemic IGF–I concentrations. Collec-tively, the studies discussed above did not differentiate between the MGF and IGF–IEa isoforms expressed in muscle. Further study is warranted to address this issue. *In vitro*, satellite cells from rats treated with trenbolone (a testosterone analog) were significantly more responsive to IGF-I than cells from control rats (Thompson *et al.* 1989), suggesting that androgens also increase the sensitivity to IGF-I, perhaps through up-regulation of the IGF receptor. Although studies confirm the existence of a pathway of androgen action through regulation of IGF–I in skeletal muscle, the extent of involvement of the androgen/IGF–I pathway in the whole process of androgen regulation of muscle cells remains unclear.

Glucocorticoid receptor Glucocorticoid hormones have profound effects on metabolism and cellular proliferation. In skeletal muscle glucocorticoids have catabolic effects on muscle (Crawford *et al.* 2003). The actions of glucocorticoids on muscle are mediated through the glucocorticoid receptor (GR) (Chen *et al.* 1997). The GR and AR are members of the nuclear receptor superfamily, and both receptors can modulate gene expression through conserved imperfect 12 bp palindromic response elements (Beato *et al.* 1989, Chen *et al.* 1997). However, although both the AR and GR can theoretically regulate the same target genes, the actions of androgens and glucocorticoids in skeletal muscle are opposing.

Androgens can offset the catabolic effects of glucocorticoids on skeletal muscle. In humans, androgen treatment increased muscle mass in men with muscle wasting due to long-term glucocorticoid therapy (Crawford *et al.* 2003). Androgen therapy in COPD patients who are on glucocorticoid treatment can partly reverse muscle wasting (Creutzberg & Schols 1999, Creutzberg *et al.* 2003). Similarly in rats, administration of glucocorticoids resulted in atropy in the diaphragm, but simultaneous administration of testosterone with glucocorticoids partially attenuated the loss in diaphragm weight (Eason *et al.* 2003).

However, the underlying mechanisms by which androgens counteract the catabolic action of glucocorticoids in muscle are not clear. Several possible mechanisms have been proposed. First, there could be binding competition between androgens and glucocorticoids for the GR (Van Balkom *et al.* 1998). *In vitro*, addition of R1881 (a synthetic androgen) in 25 molar excess decreased cortisol binding to the GR, while oxandrolone in up to 250 molar excess had no effect on cortisol binding (Zhao *et al.* 2004). This *in vitro* study suggests the possibility that some but not all androgens have the capacity to reduce the binding affinity of glucocorticoids to the GR. However, the physiological relevance of this effect is unknown.

A second possible mechanism of the androgen/glucocorticoid antagonism is that androgens could potentially reduce the expression of the GR in the muscle. Down-regulation of GR expression mediated by androgens has been observed in the hippocampal pyramidal cell layer of CA1 neurons (Kerr *et al.* 1996) and rat motor neurons (Blanco *et al.* 2002). In muscle, it has been shown that treatment of sheep with trenbolone also reduces GR expression (Sharpe *et al.* 1986).

Another possible mechanism of action is that the AR may have a dominant negative effect on GR function (Yen *et al.* 1997). Androgens may interact through their own receptor to interfere with GR transcriptional activity. One *in vitro* study (Yen *et al.* 1997) indicated that when the GR and AR are co-expressed, they can interfere with their mutual activity. In this study, the higher the ratio of AR versus GR, the greater the suppression of GR transcription that occurred. Similarly, a recent study conducted by Zhao *et al.* (2004) demonstrated significant antagonism of cortisol-induced transcriptional activation by oxandrolone in COS7 cells expressing both the AR and GR. No inhibition by oxandrolone was observed in cells expressing GR alone. These studies indicate that a mechanism of suppression of glucocorticoid actions by androgens may be via crosstalk between the AR and GR, which could occur through their ability to form heterodimers at a common DNA site (Chen *et al.* 1997).

Collectively, the counteractions of androgens on the catabolic effects of glucocorticoids could be via interaction between the AR and GR, with the ability of androgens to increase AR levels in target tissue described previously. Additionally, androgens could down-regulate GR expression, and some androgens are capable of competitively reducing the binding affinity of glucocorticoids to GR. However, further study is needed to demonstrate if any of these mechanisms occur in normal skeletal muscle, where both AR and GR are expressed.

It is worth noting that glucocorticoid excess can also impact on androgen action, and this may also play a role in the myopathy observed following glucocorticoid treatment. Evidence suggests that excessive glucocorticoid levels decrease androgen synthesis. Male patients with rheumatoid arthritis taking prednisone have lower levels of testosterone and gonadotropins compared with patients not taking prednisone (Martens *et al.* 1994). Glucocorticoids are thought to suppress gonadotropin release by acting at the level of the pituitary gland, and additionally suppress GnRH release at the hypothalamic level (Calogero *et al.* 1999, Breen *et al.* 2004). Therefore, glucocorticoids may amplify their catabolic effects via reducing androgen production, thus reducing the normal anabolic action of androgens on muscle.

Myostatin Myostatin, a member of the transforming growth factor-β superfamily, is a negative regulator of skeletal muscle mass (McPherron & Lee 1997). In adult mice, myostatin is highly expressed in muscle, with detectable levels of myostatin mRNA present in adipose tissue. Myostatin inhibits myoblast proliferation and differentiation *in vitro* (Ma *et al.* 2001, Langley *et al.* 2002), and myostatin-deficient mice have a dramatic increase in skeletal mass (McPherron & Lee 1997). Recently, there was a report of a child who has substantial muscle hypertropy with a splice site mutation of the myostatin gene (Schuelke *et al.* 2004). Collectively, these data indicate a negative role of myostatin in the regulation of muscle size (McNally 2004).

The potential regulation of myostatin by androgens has yet to be fully investigated. One study of healthy old men (Marcell *et al.* 2001) showed no significant correlation between myostatin and AR mRNAs in muscle. There was also no relationship between circulating testosterone levels and myostatin mRNA levels. Androgen response elements are present in the 5′-regulatory region of the human myostatin promoter (Ma *et al.* 2001), raising the

possibility that myostatin could be a direct target of the AR. Because of the opposing actions of androgens and myostatin in skeletal muscle, it is tempting to speculate that androgen may suppress myostatin expression or action; however, further studies are required to address their potential relationship in muscle.

Myogenic cell commitment Another potential indirect action of androgens on myoblasts is through recruitment of stem cells into the myogenic lineage. A recent *in vitro* study showed that treatment of 10T1/2 pluripotent mesenchymal cells with testosterone or DHT significantly increased the number of myogenic cells in a dose-dependent manner, while inhibiting adipogenic differentiation (Singh *et al.* 2003). This study demonstrated that these effects are mediated through an AR-dependent mechanism, because an AR antagonist blocked the actions of testosterone or DHT. This hypothesis is also supported by the fact that in humans, CD34+ interstitial, mesenchymal cells are AR positive and expression of the AR is androgen dose-dependent (Sinha-Hikim *et al.* 2004). These data demonstrate that androgens can recruit stem cells into the myogenic lineage by committing them to myogenic precursor cells (Bhasin *et al.* 2003*b*). However, it is still not known whether androgen stimulation of myogenic commitment then gives rise to satellite cells, or directly contributes to muscle formation. Moreover, the physiological significance of the effects of androgens on stem cell commitment in contributing to muscle growth and regeneration is unclear. These preliminary studies clearly indicate that further investigation into this area is warranted.

Non-genomic pathways

In contrast to the genomic pathway (minutes to hours), there is a non-genomic pathway (seconds to minutes) elicited by hormones, the effects of which cannot be abrogated by transcriptional inhibitors, and may occur without requiring the hormone to bind the intracellular receptors or the receptor to bind DNA (Cato *et al.* 2002, Simoncini & Genazzani 2003).

Although no studies have examined potential non-genomic effects of androgens on myoblasts, non-genomic pathways have been observed in rat myotubes treated with androgens (Estrada *et al.* 2000, 2003). These effects were characterized by an early increase in intracellular calcium, triggered 15–30 s after testosterone addition, preceded by an increase in inositol 1,4,5-trisphosphate (IP3) mass (Estrada *et al.* 2000). In addition to the effects on IP3, exposure of myotubes to androgens produced an early, transient, IP3-calcium-dependent increase in extracellular signal-regulated protein kinase (ERK)1/2 phosphorylation (Estrada *et al.* 2003). The use of dominant negative mutants of Ras and MEK demonstrated that androgens activate the Ras/MEK/ERK pathway (Estrada *et al.*

2003). The actions described above were not blocked by cyproterone acetate, an inhibitor of the AR, indicating that distinct non-genomic pathways exist for the action of androgens in skeletal muscle cells (Estrada *et al.* 2000, 2003).

Although some progress has been made in the study of non-genomic androgen pathways in muscle cells, physiologically relevant effects have yet to be demonstrated. This is an area for further research.

Summary

Myoblasts are myogenic precursor cells and the major source for regeneration and growth of skeletal muscle. After activation, satellite cells become myoblasts which differentiate into myotubes that contribute to muscle formation through fusion with myofibers. Myoblasts express MyoD whereas satellite cells do not express MyoD. The AR is present in skeletal muscle and, more specifically, has been found in satellite cells. Studies have confirmed that androgens increase muscle volume in humans. In animal and human studies, an increase in the number of satellite cells upon androgen treatment has been observed. Moreover, AR levels are up-regulated in satellite cells with androgen administration, which may potentially enhance the sensitivity of satellite cells to androgen treatment. However, the mechanisms by which androgens might increase satellite cell number are not known. Androgens may potentially regulate both satellite cell proliferation and differentiation. Further investigation is warranted. The non-genomic pathway in myoblasts is a new area not fully investigated. Several studies have shown activation of non-genomic pathways in myotubes. However, more research is required to understand how this pathway interacts with classic AR-mediated pathways. Furthermore, indirect pathways of androgens in satellite cells, such as through IGF-I or anti-glucocorticoid effects, also merit further investigation. There is rapidly growing interest in androgen intervention for muscle weakness in elderly people and patients with muscle wasting disease. Limited studies so far have seen positive results. However, the mechanisms by which androgens exert their effects, particularly in myoblasts and satellite cells, remain unclear. Therefore, more detailed studies are required in this area to expand our knowledge of the mechanisms of androgen action in skeletal muscle.

Acknowledgements

References

Adams GR 1998 Role of insulin-like growth factor-I in the regulation of skeletal muscle adaptation to increased loading. *Exercise and Sport Sciences Reviews* **26** 31–60.

Allen DL, Roy RR & Edgerton VR 1999 Myonuclear domains in muscle adaptation and disease. *Muscle and Nerve* **22** 1350–1360.

Antonio J, Wilson JD & George FW 1999 Effects of castration and androgen treatment on androgen–receptor levels in rat skeletal muscles. *Journal of Applied Physiology* **87** 2016–2019.

Armand O, Boutineau AM, Mauger A, Pautou MP & Kieny M 1983 Origin of satellite cells in avian skeletal muscles. *Archives d'Anatomie Microscopique et de Morphologie Experimentale* **72** 163–181.

Asakura A, Seale P, Girgis-Gabardo A & Rudnicki MA 2002 Myogenic specification of side population cells in skeletal muscle. *Journal of Cell Biology* **159** 123–134.

Beato M, Chalepakis G, Schauer M & Slater EP 1989 DNA regulatory elements for steroid hormones. *Journal of Steroid Biochemistry* **32** 737–747.

Beauchamp JR, Heslop L, Yu DS, Tajbakhsh S, Kelly RG, Wernig A, Buckingham ME, Partridge TA & Zammit PS 2000 Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells. *Journal of Cell Biology* **151** 1221–1234.

Bhasin S, Storer TW, Berman N, Callegari C, Clevenger B, Phillips J, Bunnell TJ, Tricker R, Shirazi A & Casaburi R 1996 The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. *New England Journal of Medicine* **335** 1–7.

Bhasin S, Storer TW, Berman N, Yarasheski KE, Clevenger B, Phillips J, Lee WP, Bunnell TJ & Casaburi R 1997 Testosterone replacement increases fat-free mass and muscle size in hypogonadal men. *Journal of Clinical Endocrinology and Metabolism* **82** 407–413.

Bhasin S, Storer TW, Javanbakht M, Berman N, Yarasheski KE, Phillips J, Dike M, Sinha-Hikim I, Shen R, Hays RD *et al.* 2000 Testosterone replacement and resistance exercise in HIV-infected men with weight loss and low testosterone levels. *Journal of the American Medical Association* **283** 763–770.

Bhasin S, Woodhouse L & Storer TW 2001*a* Proof of the effect of testosterone on skeletal muscle. *Journal of Endocrinology* **170** 27–38.

Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, Chen X, Yarasheski KE, Magliano L, Dzekov C *et al.* 2001*b* Testosterone dose–response relationships in healthy young men. *American Journal of Physiology – Endocrinology and Metabolism* **281** E1172–E1181.

Bhasin S, Woodhouse L & Storer TW 2003*a* Androgen effects on body composition. *Growth Hormone and IGF Research* **13** (Suppl) S63–S71.

Bhasin S, Taylor WE, Singh R, Artaza J, Sinha-Hikim I, Jasuja R, Choi H & Gonzalez-Cadavid NF 2003*b* The mechanisms of androgen effects on body composition: mesenchymal pluripotent cell as the target of androgen action. *Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* **58** M1103–M1110.

Bischoff R 1994 The satellite cell and muscle regeneration. In *Myology*, pp 97–118, 2nd edn. Ed. AG Engel. New York: McGraw-Hill, Inc.

Blanco CE, Peltz A, Staley R & Kim F 2002 Effects of pharmacologic androgen treatment duration on glucocorticoid receptor alpha immunoreactivity of lumbosacral motor neurons in the male rat. *Neuroscience* **115** 941–949.

Breen KM, Stackpole CA, Clarke IJ, Pytiak AV, Tilbrook AJ, Wagenmaker ER, Young EA & Karsch FJ 2004 Does the type II glucocorticoid receptor mediate cortisol-induced suppression in pituitary responsiveness to gonadotropin-releasing hormone? *Endocrinology* **145** 2739–2746.

Brodsky IG, Balagopal P & Nair KS 1996 Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men – a clinical research center study. *Journal of Clinical Endocrinology and Metabolism* **81** 3469–3475.

Bross R, Storer T & Bhasin S 1999 Aging and muscle loss. *Trends in Endocrinology and Metabolism* **10** 194–198.

Calogero AE, Burrello N, Bosboom AM, Garofalo MR, Weber RF & D'Agata R 1999 Glucocorticoids inhibit gonadotropin-releasing hormone by acting directly at the hypothalamic level. *Journal of Endocrinological Investigation* **22** 666–670.

Campion DR 1984 The muscle satellite cell – a review. *International Review of Cytology – A Survey of Cell Biology* **87** 225–251.

Cato AC, Nestl A & Mink S 2002 Rapid actions of steroid receptors in cellular signaling pathways. *Science's Signal Transduction Knowledge Environment* **2002** RE9.

Charge SB & Rudnicki MA 2004 Cellular and molecular regulation of muscle regeneration. *Physiological Reviews* **84** 209–238.

Charge SB, Brack AS & Hughes SM 2002 Aging-related satellite cell differentiation defect occurs prematurely after ski-induced muscle hypertrophy. *American Journal of Physiology – Cell Physiology* **283** C1228–C1241.

Cheema U, Brown R, Mudera V, Yang SY, McGrouther G & Goldspink G 2005 Mechanical signals and IGF-I gene splicing *in vitro* in relation to development of skeletal muscle. *Journal of Cell Physiology* **202** 67–75.

Chen S, Wang J, Yu G, Liu W & Pearce D 1997 Androgen and glucocorticoid receptor heterodimer formation. A possible mechanism for mutual inhibition of transcriptional activity. *Journal of Biological Chemistry* **272** 14087–14092.

Cornelison DD & Wold BJ 1997 Single-cell analysis of regulatory gene expression in quiescent and activated mouse skeletal muscle satellite cells. *Developmental Biology* **191** 270–283.

Crawford BA, Liu PY, Kean MT, Bleasel JF & Handelsman DJ 2003 Randomized placebo-controlled trial of androgen effects on muscle and bone in men requiring long-term systemic glucocorticoid treatment. *Journal of Clinical Endocrinology and Metabolism* **88** 3167–3176.

Creutzberg EC & Schols AM 1999 Anabolic steroids. *Current Opinion in Clinical Nutrition and Metabolic Care* **2** 243–253.

Creutzberg EC, Wouters EF, Mostert R, Pluymers RJ & Schols AM 2003 A role for anabolic steroids in the rehabilitation of patients with COPD? A double-blind, placebo-controlled, randomized trial. *Chest* **124** 1733–1742.

De Angelis L, Berghella L, Coletta M, Lattanzi L, Zanchi M, Cusella-De Angelis MG, Ponzetto C & Cossu G 1999 Skeletal myogenic progenitors originating from embryonic dorsal aorta coexpress endothelial and myogenic markers and contribute to postnatal muscle growth and regeneration. *Journal of Cell Biology* **147** 869–878.

Debigare R, Marquis K, Cote CH, Tremblay RR, Michaud A, LeBlanc P & Maltais F 2003 Catabolic/anabolic balance and muscle wasting in patients with COPD. *Chest* **124** 83–89.

Doumit ME, Cook DR & Merkel RA 1996 Testosterone up-regulates androgen receptors and decreases differentiation of porcine myogenic satellite cells *in vitro*. *Endocrinology* **137** 1385–1394.

Eason JM, Dodd SL & Powers SK 2003 Use of anabolic steroids to attenuate the effects of glucocorticoids on the rat diaphragm. *Physical Therapy* **83** 29–36.

Estrada M, Liberona JL, Miranda M & Jaimovich E 2000 Aldosterone- and testosterone-mediated intracellular calcium response in skeletal muscle cell cultures. *American Journal of Physiology – Endocrinology and Metabolism* **279** E132–E139.

Estrada M, Espinosa A, Muller M & Jaimovich E 2003 Testosterone stimulates intracellular calcium release and mitogen-activated protein kinases via a G protein-coupled receptor in skeletal muscle cells. *Endocrinology* **144** 3586–3597.

Evans RM 1988 The steroid and thyroid hormone receptor superfamily. *Science* **240** 889–895.

Ferrando AA, Sheffield-Moore M, Wolf SE, Herndon DN & Wolfe RR 2001 Testosterone administration in severe burns ameliorates muscle catabolism. *Critical Care Medicine* **29** 1936–1942.

Ferrando AA, Sheffield-Moore M, Yeckel CW, Gilkison C, Jiang J, Achacosa A, Lieberman SA, Tipton K, Wolfe RR & Urban RJ 2002 Testosterone administration to older men improves muscle function: molecular and physiological mechanisms. *American Journal of Physiology – Endocrinology and Metabolism* **282** E601–E607.

Florini JR, Ewton DZ & Coolican SA 1996 Growth hormone and the insulin-like growth factor system in myogenesis. *Endocrine Reviews* **17** 481–517.

Freedman LP 1992 Anatomy of the steroid receptor zinc finger region. *Endocrine Reviews* **13** 129–145.

Goldspink G & Harridge SD 2004 Growth factors and muscle ageing. *Experimental Gerontology* **39** 1433–1438.

Grounds MD, White JD, Rosenthal N & Bogoyevitch MA 2002 The role of stem cells in skeletal and cardiac muscle repair. *Journal of Histochemistry and Cytochemistry* **50** 589–610.

Gruenewald DA & Matsumoto AM 2003 Testosterone supplementation therapy for older men: potential benefits and risks. *Journal of the American Geriatric Society* **51** 101–115; discussion 115.

Gussoni E, Soneoka Y, Strickland CD, Buzney EA, Khan MK, Flint AF, Kunkel LM & Mulligan RC 1999 Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature* **401** 390–394.

Hawke TJ & Garry DJ 2001 Myogenic satellite cells: physiology to molecular biology. *Journal of Applied Physiology* **91** 534–551.

Hill M, Wernig A & Goldspink G 2003 Muscle satellite (stem) cell activation during local tissue injury and repair. *Journal of Anatomy* **203** 89–99.

Joubert Y & Tobin C 1989 Satellite cell proliferation and increase in the number of myonuclei induced by testosterone in the levator ani muscle of the adult female rat. *Developmental Biology* **131** 550–557.

Joubert Y & Tobin C 1995 Testosterone treatment results in quiescent satellite cells being activated and recruited into cell cycle in rat levator ani muscle. *Developmental Biology* **169** 286–294.

Joubert Y, Tobin C & Lebart MC 1994 Testosterone-induced masculinization of the rat levator ani muscle during puberty. *Developmental Biology* **162** 104–110.

Kadi F, Eriksson A, Holmner S & Thornell LE 1999 Effects of anabolic steroids on the muscle cells of strength-trained athletes. *Medicine and Science in Sports and Exercise* **31** 1528–1534.

Kadi F, Bonnerud P, Eriksson A & Thornell LE 2000 The expression of androgen receptors in human neck and limb muscles: effects of training and self-administration of androgenic-anabolic steroids. *Histochemistry and Cell Biology* **113** 25–29.

Kadi F, Charifi N, Denis C & Lexell J 2004 Satellite cells and myonuclei in young and elderly women and men. *Muscle and Nerve* **29** 120–127.

Kamanga-Sollo E, Pampusch MS, Xi G, White ME, Hathaway MR & Dayton WR 2004 IGF-I mRNA levels in bovine satellite cell cultures: effects of fusion and anabolic steroid treatment. *Journal of Cell Physiology* **201** 181–189.

Kerr JE, Beck SG & Handa RJ 1996 Androgens modulate glucocorticoid receptor mRNA, but not mineralocorticoid receptor mRNA levels, in the rat hippocampus. *Journal of Neuroendocrinology* **8** 439–447.

Krieg M 1976 Characterization of the androgen receptor in the skeletal muscle of the rat. *Steroids* **28** 261–274.

Langley B, Thomas M, Bishop A, Sharma M, Gilmour S & Kambadur R 2002 Myostatin inhibits myoblast differentiation by down-regulating MyoD expression. *Journal of Biological Chemistry* **277** 49831–49840.

Layne MD & Farmer SR 1999 Tumor necrosis factor-alpha and basic fibroblast growth factor differentially inhibit the insulin-like growth factor-I induced expression of myogenin in C2C12 myoblasts. *Experimental Cell Research* **249** 177–187.

Lee DK 2002 Androgen receptor enhances myogenin expression and accelerates differentiation. *Biochemical and Biophysical Research Communications* **294** 408–413.

Lee WJ, Thompson RW, McClung JM & Carson JA 2003 Regulation of androgen receptor expression at the onset of functional overload in rat plantaris muscle. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* **285** R1076–R1085.

Lewis MI, Horvitz GD, Clemmons DR & Fournier M 2002 Role of IGF-I and IGF-binding proteins within diaphragm muscle in modulating the effects of nandrolone. *American Journal of Physiology – Endocrinology and Metabolism* **282** E483–E490.

Ma K, Mallidis C, Artaza J, Taylor W, Gonzalez-Cadavid N & Bhasin S 2001 Characterization of 5′-regulatory region of human myostatin gene: regulation by dexamethasone *in vitro*. *American Journal of Physiology – Endocrinology and Metabolism* **281** E1128–E1136.

McNally EM 2004 Powerful genes – myostatin regulation of human muscle mass. *New England Journal of Medicine* **350** 2642–2644.

McPherron AC & Lee SJ 1997 Double muscling in cattle due to mutations in the myostatin gene. *PNAS* **94** 12457–12461.

Marcell TJ, Harman SM, Urban RJ, Metz DD, Rodgers BD & Blackman MR 2001 Comparison of GH, IGF-I, and testosterone with mRNA of receptors and myostatin in skeletal muscle in older men. *American Journal of Physiology – Endocrinology and Metabolism* **281** E1159–E1164.

Martens HF, Sheets PK, Tenover JS, Dugowson CE, Bremner WJ & Starkebaum G 1994 Decreased testosterone levels in men with rheumatoid arthritis: effect of low dose prednisone therapy. *Journal of Rheumatology* **21** 1427–1431.

Mauras N, Hayes V, Welch S, Rini A, Helgeson K, Dokler M, Veldhuis JD & Urban RJ 1998 Testosterone deficiency in young men: marked alterations in whole body protein kinetics, strength, and adiposity. *Journal of Clinical Endocrinology and Metabolism* **83** 1886–1892.

Michel G & Baulieu EE 1980 Androgen receptor in rat skeletal muscle: characterization and physiological variations. *Endocrinology* **107** 2088–2098.

Mooradian AD, Morley JE & Korenman SG 1987 Biological actions of androgens. *Endocrine Reviews* **8** 1–28.

Mulvaney DR, Marple DN & Merkel RA 1988 Proliferation of skeletal muscle satellite cells after castration and administration of testosterone propionate. *Proceedings of the Society for Experimental Biology and Medicine* **188** 40–45.

Nnodim JO 1999*a* Effect of aging on myofiber and satellite cell numbers in rat levator ani muscle. *FASEB Journal* **13 (Part 2 suppl)** A688.

Nnodim JO 1999*b* Quantitative study of the effects of denervation and castration on the levator ani muscle of the rat. *Anatomical Record* **255** 324–333.

Nnodim JO 2000 Satellite cell numbers in senile rat levator ani muscle. *Mechanisms of Ageing and Development* **112** 99–111.

Nnodim JO 2001 Testosterone mediates satellite cell activation in denervated rat levator ani muscle. *Anatomical Record* **263** 19–24.

Powers ML & Florini JR 1975 A direct effect of testosterone on muscle cells in tissue culture. *Endocrinology* **97** 1043–1047.

Renault V, Piron-Hamelin G, Forestier C, DiDonna S, Decary S, Hentati F, Saillant G, Butler-Browne GS & Mouly V 2000 Skeletal muscle regeneration and the mitotic clock. *Experimental Gerontology* **35** 711–719.

Sattler FR, Jaque SV, Schroeder ET, Olson C, Dube MP, Martinez C, Briggs W, Horton R & Azen S 1999 Effects of pharmacological doses of nandrolone decanoate and progressive resistance training in immunodeficient patients infected with human immunodeficiency virus. *Journal of Clinical Endocrinology and Metabolism* **84** 1268–1276.

Schroeder ET, Terk M & Sattler FR 2003*a* Androgen therapy improves muscle mass and strength but not muscle quality: results from two studies. *American Journal of Physiology – Endocrinology and Metabolism* **285** E16–E24.

Schroeder ET, Singh A, Bhasin S, Storer TW, Azen C, Davidson T, Martinez C, Sinha-Hikim I, Jaque SV, Terk M *et al.* 2003*b* Effects

of an oral androgen on muscle and metabolism in older, community-dwelling men. *American Journal of Physiology – Endocrinology and Metabolism* **284** E120–E128.

Schuelke M, Wagner KR, Stolz LE, Hubner C, Riebel T, Komen W, Braun T, Tobin JF & Lee SJ 2004 Myostatin mutation associated with gross muscle hypertrophy in a child. *New England Journal of Medicine* **350** 2682–2688.

Schultz E 1996 Satellite cell proliferative compartments in growing skeletal muscles. *Developmental Biology* **175** 84–94.

Seale P & Rudnicki MA 2000 A new look at the origin, function, and stem-cell status of muscle satellite cells. *Developmental Biology* **218** 115–124.

Seale P, Asakura A & Rudnicki MA 2001 The potential of muscle stem cells. *Developmental Cell* **1** 333–342.

Sharpe PM, Buttery PJ & Haynes NB 1986 The effect of manipulating growth in sheep by diet or anabolic agents on plasma cortisol and muscle glucocorticoid receptors. *British Journal of Nutrition* **56** 289–304.

Simoncini T & Genazzani AR 2003 Non-genomic actions of sex steroid hormones. *European Journal of Endocrinology* **148** 281–292.

Singh R, Artaza JN, Taylor WE, Gonzalez-Cadavid NF & Bhasin S 2003 Androgens stimulate myogenic differentiation and inhibit adipogenesis in C3H 10T1/2 pluripotent cells through an androgen receptor-mediated pathway. *Endocrinology* **144** 5081–5088.

Sinha-Hikim I, Artaza J, Woodhouse L, Gonzalez-Cadavid N, Singh AB, Lee MI, Storer TW, Casaburi R, Shen R & Bhasin S 2002 Testosterone-induced increase in muscle size in healthy young men is associated with muscle fiber hypertrophy. *American Journal of Physiology – Endocrinology and Metabolism* **283** E154–E164.

Sinha-Hikim I, Roth SM, Lee MI & Bhasin S 2003 Testosterone-induced muscle hypertrophy is associated with an increase in satellite cell number in healthy, young men. *American Journal of Physiology – Endocrinology and Metabolism* **285** E197–E205.

Sinha-Hikim I, Taylor WE, Gonzalez-Cadavid NF, Zheng W & Bhasin S 2004 Androgen receptor in human skeletal muscle and cultured muscle satellite cells: up-regulation by androgen treatment. *Journal of Clinical Endocrinology and Metabolism* **89** 5245–5255.

Snyder PJ 2001 Effects of age on testicular function and consequences of testosterone treatment. *Journal of Clinical Endocrinology and Metabolism* **86** 2369–2372.

Thompson SH, Boxhorn LK, Kong WY & Allen RE 1989 Trenbolone alters the responsiveness of skeletal muscle satellite cells to fibroblast growth factor and insulin-like growth factor I. *Endocrinology* **124** 2110–2117.

Van Balkom RH, Dekhuijzen PN, Folgering HT, Veerkamp JH, Van Moerkerk HT, Fransen JA & Van Herwaarden CL 1998 Anabolic steroids in part reverse glucocorticoid-induced alterations in rat diaphragm. *Journal of Applied Physiology* **84** 1492–1499.

Wolf SE, Thomas SJ, Dasu MR, Ferrando AA, Chinkes DL, Wolfe RR & Herndon DN 2003 Improved net protein balance, lean mass, and gene expression changes with oxandrolone treatment in the severely burned. *Annals of Surgery* **237** 801–810.

Wong CI, Zhou ZX, Sar M & Wilson EM 1993 Steroid requirement for androgen receptor dimerization and DNA binding. Modulation by intramolecular interactions between the NH2-terminal and steroid-binding domains. *Journal of Biological Chemistry* **268** 19004–19012.

Yang SY & Goldspink G 2002 Different roles of the IGF-IEc peptide (MGF) and mature IGF-I in myoblast proliferation and differentiation. *FEBS Letters* **522** 156–160.

Yeh SS, DeGuzman B & Kramer T 2002 Reversal of COPD-associated weight loss using the anabolic agent oxandrolone. *Chest* **122** 421–428.

Yen PM, Liu Y, Palvimo JJ, Trifiro M, Whang J, Pinsky L, Janne OA & Chin WW 1997 Mutant and wild-type androgen receptors exhibit cross-talk on androgen-, glucocorticoid-, and progesterone-mediated transcription. *Molecular Endocrinology* **11** 162–171.

Yialamas MA & Hayes FJ 2003 Androgens and the ageing male and female. *Best Practice and Research. Clinical Endocrinology and Metabolism* **17** 223–236.

Yin D, Gao W, Kearbey JD, Xu H, Chung K, He Y, Marhefka CA, Veverka KA, Miller DD & Dalton JT 2003 Pharmacodynamics of selective androgen receptor modulators. *Journal of Pharmacology and Experimental Therapeutics* **304** 1334–1340.

Zammit P & Beauchamp J 2001 The skeletal muscle satellite cell: stem cell or son of stem cell? *Differentiation* **68** 193–204.

Zammit PS, Golding JP, Nagata Y, Hudon V, Partridge TA & Beauchamp JR 2004 Muscle satellite cells adopt divergent fates: a mechanism for self-renewal? *Journal of Cell Biology* **166** 347–357.

Zhao J, Bauman WA, Huang R, Caplan AJ & Cardozo C 2004 Oxandrolone blocks glucocorticoid signaling in an androgen receptor-dependent manner. *Steroids* **69** 357–366.