Androgen regulation of satellite cell function

Yue Chen, Jeffrey D Zajac and Helen E MacLean
Department of Medicine, University of Melbourne, Austin Health, Heidelberg, Victoria 3084, Australia
(Requests for offprints should be addressed to Helen E MacLean; Email: hmaclean@unimelb.edu.au)

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.

Introduction

With increasing age, skeletal muscle mass is lost, and aging atrophy 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 (Brodsy et al. 1996, Bhasin et al. 1997, 2001a,b, Mauras et al. 1998, Sinha–Hikim et al. 2002). In vivo, androgens can increase skeletal muscle mass (NNodim 1999b). 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 (Moordadian 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. 2003a).

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.
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 myofibers (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.
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. 2000). 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).

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. 2000). 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).

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, 2001b). In addition, animal studies also show anabolic effects of androgen on muscle size and strength (Antonio et al. 1999, Nnodim 1999). 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.

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 hypertrophy (Bhasin et al. 2001b). The treatment was associated with a dose-dependent increase in myonuclear marker expression and an increase in satellite cell number (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.

Physiology

There is functional evidence that satellite cells are a heterogeneous population. In vivo, 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 G0 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 microenvironment required for the efficient activation and differentiation of satellite cells (Renault et al. 2000, Seale et al. 2001, Charge et al. 2002).

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 hypertrophy (Bhasin et al. 2001b). The treatment was associated with a dose-dependent increase in myonuclear marker expression and an increase in satellite cell number (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.
number (Sinha-Hikim \textit{et al.} 2002). Therefore, testosterone increases skeletal muscle size partly by inducing muscle fiber hypertrophy.

**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 \textit{et al.} 2003b) 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 \textit{et al.} 2003a), 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 \textit{et al.} 2000). Men treated with testosterone experienced significant increases in maximum voluntary muscle strength, associated with an increase in muscle volume (Sattler \textit{et al.} 1999, Bhasin \textit{et al.} 2000). Weight loss in chronic obstructive pulmonary disease (COPD) is associated with skeletal muscle dysfunction and increased mortality (Yeh \textit{et al.} 2002). It has been shown that there is a greater prevalence of hypogonadism in patients with COPD compared with normal men (Debigare \textit{et al.} 2003). Improvements in muscle function and muscle size have been observed in COPD patients treated with nandrolone (Creutzberg & Schols 1999, Creutzberg \textit{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 \textit{et al.} 2001, Wolf \textit{et al.} 2003).

**Androgens in athletic performance** There is agreement that testosterone supplementation increases maximal voluntary strength of muscle (Sattler \textit{et al.} 1999, Bhasin \textit{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 \textit{et al.} 1999). Therefore, it is not surprising that the abuse of anabolic steroids is most prevalent among power lifters. Testosterone may also enhance recovery from exercise and improve explosive powers (Bhasin \textit{et al.} 2001a), 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). \textit{In vitro}, the AR protein has been demonstrated in porcine satellite cells (Doumit \textit{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 levator ani muscle satellite cell number not only in males but also in females (Joubert \textit{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 \textit{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 \textit{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 \textit{et al.} 1999, Sinha-Hikim \textit{et al.} 2003). This observation is in accordance with a previous study in rats (Joubert \textit{et al.} 1994) that showed that muscle fiber hypertrophy 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 \textit{et al.} 1999).
Table 1 Summary of in vitro studies examining effects of androgens on proliferation and differentiation of satellite cells or myoblasts

<table>
<thead>
<tr>
<th>Target cells</th>
<th>Androgen treatment</th>
<th>Effects on proliferation</th>
<th>Effects on differentiation</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary myoblasts (rat)</td>
<td>Testosterone</td>
<td>Enhanced proliferation</td>
<td>Not examined</td>
<td>Powers &amp; Florini 1975</td>
</tr>
<tr>
<td>Satellite cells (rat)</td>
<td>Trenbolone</td>
<td>No effect</td>
<td>Not examined</td>
<td>Thompson et al. 1989</td>
</tr>
<tr>
<td>Satellite cells (pig)</td>
<td>Testosterone</td>
<td>No effect</td>
<td>Reduction in differentiation</td>
<td>Doumit et al. 1996</td>
</tr>
<tr>
<td>C2C12 cells (mouse myoblast cell line)</td>
<td>Testosterone</td>
<td>No effect</td>
<td>Increase in differentiation</td>
<td>Lee 2002</td>
</tr>
</tbody>
</table>

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-Ie (Goldspink & Harridge 2004). The former is mechanosensitive and autocrine, while the latter is similar to the systemic or liver type of IGF-I.
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 myoblast 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). 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 hormone (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. Collectively, 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 atrophy 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 hypertrophy 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. 2003b). 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**

Y C is an Australian National Health and Medical Research Council (NHMRC) Postgraduate Scholarship recipient #310636. H E M is supported by NHMRC Career Development Awards Fellowship #359226. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.
References


Androgen actions in satellite cells


---


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

---

*Journal of Endocrinology (2005) 186, 21–31*


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.


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


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


Received in final form 13 April 2005
Accepted 22 April 2005
Made available online as an Accepted Preprint 5 May 2005