Targeting muscle signaling pathways to minimize adverse effects of androgen deprivation

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Abstract

Androgen deprivation therapy (ADT) is a highly effective treatment used in ~30% of men with prostate cancer. Adverse effects of ADT on muscle are significant with consistent losses in muscle mass. However, effects of ADT on muscle strength and physical function, of most relevance to the patient, are less well understood. This is in part due to the fact that muscle effects of ADT at the cellular, genetic and protein level, critical to the understanding of the pathophysiology of sarcopenia, have come into focus only recently. This review highlights the complexity of androgen-dependent signaling in muscle with an emphasis on recent findings in the regulation of muscle growth and muscle atrophy pathways. Furthermore, the effects of ADT and testosterone on skeletal muscle histology, gene expression and protein transcription are discussed. A better mechanistic understanding of the regulation of muscle mass and function by androgens should not only pave the way for developing targeted promyogenic interventions for men with prostate cancer receiving ADT but also may have wider implications for age-associated sarcopenia in the general population.

Key Words

- ▶ androgen deprivation
- ▶ histology
- ▶ skeletal muscle
- ▶ sarcopenia
- ▶ dynapenia

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Introduction

Prostate cancer is the most common cancer affecting men worldwide, with a particularly high prevalence in developed countries (Basaria & Bhasin 2012, Grossmann *et al.* 2013). Despite a relatively low case-fatality rate, prostate cancer represents the second largest cause of cancer deaths in men in affluent countries, primarily because of the high overall prevalence (Grossmann *et al.* 2013). Due to the important role of androgen-receptor signaling in the development of prostate cancer, androgen deprivation therapy (ADT) is commonly used for both locally advanced and metastatic disease; however, benefits are accompanied by a variety of side effects due to the widespread expression of sex steroid receptors (Basaria & Bhasin 2012, Cheung *et al.* 2014). Adverse effects include

loss of muscle, accelerated bone loss and increased adiposity as well as anemia, hot flushes and sexual dysfunction (Basaria & Bhasin 2012, Dubois *et al.* 2012, Chang *et al.* 2013, Grossmann *et al.* 2013, Serra *et al.* 2013a, White *et al.* 2013). Consequently, ADT is associated with increased morbidity, frailty and reduced quality of life (Alibhai *et al.* 2010). Increased cardiometabolic risk is a major concern, to which endocrine actions of adipose tissue and skeletal muscle may contribute in part via secretion of myokines and adipokines that regulate body composition, insulin sensitivity and atherosclerosis (Argiles *et al.* 2005, Basaria & Bhasin 2012, Grossmann *et al.* 2013). As first postulated by Basaria and Bhasin (2012), the metabolic derangements seen in patients

receiving ADT likely represent disruption of the skeletal muscle-metabolism axis, whereby loss of anabolic signaling has implications far beyond a loss of muscle mass and strength. Thus, interventions targeting skeletal muscle signaling pathways may be beneficial in terms of not only improving skeletal muscle mass and strength (and quality of life) but also reducing cardiometabolic risk (Basaria & Bhasin 2012, Grossmann *et al.* 2013). Testosterone and perhaps its derivative estradiol are among the key mediators in these processes; however, the specific mechanisms and interactions are not fully elucidated (Wu & von Eckardstein 2003, Finkelstein *et al.* 2013). Testosterone administration has a consistent, dose-dependent anabolic effect in a number of populations, including androgen-deficient and eugonadal young men, older men and men with chronic diseases such as HIV (Herbst & Bhasin 2004, Bhasin *et al.* 2006, Sinha-Hikim *et al.* 2006, Miller 2009, Jones *et al.* 2010, Dubois *et al.* 2012, Jasuja *et al.* 2014). However, benefits of testosterone supplementation on physical function and subsequent quality of life are more difficult to define, despite being the most relevant factor for the patient (Herbst & Bhasin 2004, Bhasin *et al.* 2006, Cheung *et al.* 2014). Similarly, although there is marked loss of muscle mass with ADT, the effect of androgen deprivation on strength and physical performance is less well understood (Cheung *et al.* 2014). In part, this is because few studies have investigated the effects of androgen deprivation on skeletal muscle at the cellular and genetic levels. A better understanding of the basic mechanisms underlying testosterone's effect on skeletal muscle, particularly histological and molecular changes, and testosterone-regulated genes should not only offer a better understanding of its role in muscle function and physical performance but also guide further strategies to mitigate adverse muscle effects of androgen deprivation.

Effects of ADT on skeletal muscle morphology

There is a lack of consensus regarding the definition of sarcopenia. The term typically describes the loss of muscle mass, strength and functional impairment that occurs with aging (Mobasheri & Mendes 2013). Controversy exists, however, as the relationship between muscle mass and muscle strength is not entirely clear, with some studies demonstrating that declining muscle mass explains <10% of the reduction in muscle strength in older adults (Hughes *et al.* 2001, Goodpaster *et al.* 2006, Clark & Manini 2012, Mobasheri & Mendes 2013, McGregor *et al.* 2014, Scott *et al.* 2014). Muscles are regulated by motor neurons and it is difficult to separate

primary muscle pathology from central or peripheral nervous system pathology, which are major contributors to the loss of muscle strength (Clark & Manini 2012, Russ *et al.* 2012, Mobasheri & Mendes 2013). Consequently, it has been suggested that the loss of muscle mass and of muscle strength should be defined separately, with sarcopenia referring to the loss of muscle mass specifically, and the term 'dynapenia' proposed to define the loss of muscle strength (Clark & Manini 2008, Scott *et al.* 2014). This has functional implications, as dynapenia is thought to be associated with impaired physical function more strongly than sarcopenia (Scott *et al.* 2014). Additionally, obesity is often associated with both conditions and this association can be described as sarcopenic obesity (obesity and loss of muscle mass) or dynapenic obesity (obesity and loss of muscle strength), with dynapenic obesity thought to have the greatest negative impact on functional status (Scott *et al.* 2014).

In men undergoing ADT, the specific effects on muscle have not been fully elucidated. At the macroscopic level, it is well known that men undergoing ADT lose muscle mass and gain fat, i.e., develop sarcopenic obesity (Bhasin *et al.* 2006, Galvao *et al.* 2009, Miller 2009, White *et al.* 2013, Girard *et al.* 2014). This change in body composition is disproportionate: lean body mass is reduced by ~2–4%, and fat mass is increased by ~10–20% in the first 12 months following the commencement of ADT (Dubois *et al.* 2012, Cheung *et al.* 2014). This is associated with a decrease in strength, predominantly in the upper body (i.e., hand grip strength); however, the data is not all concordant, with some studies demonstrating inconsistent changes in strength (Bhasin *et al.* 2006, Cheung *et al.* 2014). Effects on physical function are even more variable, and this may be due to the insensitivity to detect changes in previously used tests (i.e., short physical performance battery, frailty scores, timed up and go) and variable outcome measures among studies that may not be adequately testing participants to maximal capacity (Bhasin *et al.* 2006). Additionally, it may reflect a lack of impact of androgen deficiency on some of the other contributors to impaired muscle function, such as neuromuscular integrity (Mobasheri & Mendes 2013).

Skeletal muscle histological changes

There has been limited analysis of the effect of ADT on skeletal muscle at the histological level, and current literature consists of a small number of animal studies. More evidence exists regarding the effects of testosterone administration, which allows some possible inferences.

Effects of androgen deprivation: humans

Currently there has not been any specific study into the effect of ADT on human skeletal muscle histology. Therefore, it is not yet known whether ADT may preferentially cause atrophy in certain muscle fiber types, i.e., as seen in disease states such as chronic obstructive pulmonary disease and congestive heart failure in which a type I (slow twitch) to type II (fast twitch) fiber-type shift is seen in limb muscles (Bhasin *et al.* 2006, Ciciliot *et al.* 2013). Aging in men (a relatively hypoandrogenic state) is typically described as associated with preferential atrophy of type II fibers (Corbu *et al.* 2010, Serra *et al.* 2013b). Despite widespread acceptance of this hypothesis in the current literature, there is a lack of robust evidence to support this association. These assumptions are primarily based on the analysis of one individual, which relied on qualitative and inferential data, conducted more than 20 years ago (Lexell 1995). A systematic review conducted in 2007 highlighted the lack of clear evidence underlying the current consensus (Brunner *et al.* 2007). A more recent review concluded that a loss of type II fibers is not consistently seen with aging (Purves-Smith *et al.* 2014). Although the castrate levels of testosterone resulting from ADT are not typically seen in aging men, a gradual decline in testosterone levels is observed, and therefore men undertaking ADT may potentially be considered an accelerated model of aging sarcopenia-dynapenia (Bhasin *et al.* 2006, O'Connell *et al.* 2011, Basaria & Bhasin 2012). Given the clinical importance of impaired muscle function in an aging population, it is clear that a better understanding of the effect of aging on skeletal muscle histology is required.

Mechanisms leading to muscle atrophy with ADT are poorly understood. In addition to changes in fiber size and possibly fiber type, changes in intramuscular fat content may occur in androgen-deficient states. Aging has been associated with an increased infiltration of muscle by adipose tissue, known as myosteatosis, which is linked to insulin resistance and increased cardiometabolic risk, although the functional impact on the skeletal muscle is not clear (Chang *et al.* 2013, Kelley & Goodpaster 2015). There is significant cross-talk between fat and muscle, and it is possible that proinflammatory adipokines contribute to adverse effects on muscle. It is not known whether myosteatosis occurs in men receiving ADT; however, a recent study using computed tomography demonstrated reduced muscle attenuation (an indirect measure of intramuscular lipid content) following 3 months of ADT (Chang *et al.* 2013). This has not yet been demonstrated histologically in men taking ADT.

Furthermore, it is possible that testosterone (and, therefore, androgen deprivation) may also affect other cell types within skeletal muscle, such as fibroblasts, blood vessels and motor neurons (MacLean *et al.* 2008, Dubois *et al.* 2012, Serra *et al.* 2013b). Fibroblasts have been implicated in the regulation of satellite cell activation and muscle regeneration (Serra *et al.* 2013b). Further research is required to investigate the impact of androgen deprivation on these cell types as they may play an important role in muscle atrophy/hypertrophy signaling pathways.

Effects of androgen deprivation: animal models

Muscle effects of androgen deprivation have been investigated in animal models, using orchiectomized or androgen receptor knockout (ARKO) rodents. Experimental androgen deprivation using either of these models typically results in a rapid loss of muscle mass and increased fat stores (Oner *et al.* 2008, White *et al.* 2013, De Naeyer *et al.* 2014).

In rodents, castration has been shown to result in a decreased myofiber cross-sectional area (CSA) in both type I and II fibers, which is consistent with the opposing effects seen with testosterone replacement (Oner *et al.* 2008, White *et al.* 2013, Rana *et al.* 2014a). Decreased contractile strength (i.e., measured by twitch force in mice) has also been variably demonstrated (White *et al.* 2013, De Naeyer *et al.* 2014). Furthermore, these studies have typically shown a rapid reversal of the castration-induced changes following the administration of exogenous testosterone, giving further weight to the causal relationship between androgen deprivation and muscle atrophy (Oner *et al.* 2008, Serra *et al.* 2013a, De Naeyer *et al.* 2014).

In addition, changes in morphology have been noted, such as irregular Z lines, loss of the lamina externa and glycogen clusters under the sarcomeres in androgen-deprived rats (Sinha-Hikim *et al.* 2006, Oner *et al.* 2008, White *et al.* 2013). Mitochondrial structural changes (i.e., dissolved cristae) and swelling have also been observed (Boissonneault 2001, Oner *et al.* 2008, Sinha *et al.* 2014).

There are some problems with generalizing these results to humans. The rodent studies used a large variety of skeletal muscle groups, and not all muscles respond to androgen deprivation or testosterone administration in the same way (Ustunel *et al.* 2003, Serra *et al.* 2013b, De Naeyer *et al.* 2014, Rana *et al.* 2014b). The levator ani is highly responsive to androgen deprivation, reducing in mass by up to 85% after orchiectomy (Oner *et al.* 2008,

Rana *et al.* 2014b). In contrast, limb muscles (i.e., triceps, extensor digitorum longus) are less responsive (Ustunel *et al.* 2003, Serra *et al.* 2013b, De Naeyer *et al.* 2014). In humans, the relative androgen sensitivity of muscle groups such as levator ani is not known, therefore, responsiveness may yield similar patterns or be entirely different. It has been suggested that the variability of androgen responsiveness between different muscle groups may result from differing levels of androgen receptor (AR) expression, but other factors, i.e., fiber-type diversity, likely contribute to this substantial heterogeneity (Dubois *et al.* 2012, Ciciliot *et al.* 2013, De Naeyer *et al.* 2014). Further research is required to elucidate whether androgen sensitivity varies significantly between muscle groups in humans, and if so, the possible mechanisms underlying any differences.

Effects of testosterone administration

Contrastingly, there have been a number of studies examining the effects of testosterone administration on skeletal muscle histology. Multiple studies (animal and human) have found that the increase in muscle mass induced by testosterone is at least partly due to muscle fiber hypertrophy, demonstrated by a dose-dependent increase in the myonuclear number and CSA of both type I and type II fibers with testosterone administration (Bhasin *et al.* 2003, Miller 2009). Relative proportions of type I, type II and mixed fibers, as well as total number of muscle fibers, are thought to remain the same (Sinha-Hikim *et al.* 2002, 2006, Herbst & Bhasin 2004).

Satellite cells also play a significant role, with testosterone administration increasing satellite cell number in animal models and both elderly and young men (Bhasin *et al.* 2003, Sinha-Hikim *et al.* 2003, 2006, Oner *et al.* 2008, Serra *et al.* 2013b, Sipila *et al.* 2013). Satellite cells are the quiescent precursor to myoblasts, the committed myogenic cells that proliferate leading to muscle growth and regeneration (Chen *et al.* 2005). Satellite cell activity can be measured by two markers: proliferating cell nuclear antigen (PCNA), an immunohistochemical marker of entry into the cell cycle, and myogenin, a myogenic regulatory factor (MRF) that regulates terminal differentiation of myoblasts into myotubes and myofibers (Sinha-Hikim *et al.* 2003, 2006, Chen *et al.* 2005, Dubois *et al.* 2012). These findings of PNCA+ and myogenin+ satellite cells, along with findings of an increased expression of notch, a key signaling molecule in satellite cell proliferation, give weight to the hypothesis that testosterone exerts its

anabolic effects by promoting satellite cell replication and activation, resulting in an increased number of myogenically committed satellite cells (Bhasin *et al.* 2003, Sinha-Hikim *et al.* 2006, Brown *et al.* 2009). In addition to an increased number and activity of satellite cells, ultrastructural changes within the satellite cells themselves have been noted, such as increased mean cell area and mean mitochondrial area, a decreased nuclear-cytoplasmic ratio and structural changes including a larger amount of endoplasmic reticulum and more pinocytic vesicles (Bhasin *et al.* 2003, Sinha-Hikim *et al.* 2003, Herbst & Bhasin 2004).

Testosterone administration has been demonstrated to induce the activation of myogenic cells, leading to an increased myonuclear number and muscle hypertrophy, without changing fiber type proportions, and it remains to be seen whether the converse is seen in ADT (Sinha-Hikim *et al.* 2002, 2006, Herbst & Bhasin 2004).

Skeletal muscle signaling pathways

Skeletal muscle mass is generally considered to be the result of a dynamic balance between signaling pathways that regulate muscle protein synthesis and degradation (see Fig. 1) (Serra *et al.* 2013a, Rodriguez *et al.* 2014). Therefore, it is logical that the loss of muscle mass associated with androgen deprivation may result from decreased protein synthesis, increased degradation or some combination of both (Serra *et al.* 2013a). These signaling pathways are complex and not fully understood, but it appears that testosterone may be a key modulator in the cross-communication between pathways (Dubois *et al.* 2012). It is generally accepted that androgens, the growth hormone/insulin-like growth factor-1 (GH/IGF1) axis and follistatin (an extracellular protein) all activate anabolic pathways, a process that results in the growth of muscle mass (Basaria & Bhasin 2012). In the opposing process, myostatin (a potent negative regulator of muscle mass development and a member of the transforming growth factor β superfamily) and ubiquitin ligases regulate pathways that result in skeletal muscle degradation (Basaria & Bhasin 2012). These opposing pathways and the considerable cross-communication between them are summarized schematically in Fig. 1.

Cellular signaling pathways mediating the anabolic actions of testosterone

The role of testosterone in skeletal muscle signaling is varied and complex, with many aspects remaining poorly

Figure 1

Schematic illustration of interaction of testosterone with the skeletal muscle signaling pathways. There is substantial cross-communication between anabolic and catabolic pathways. Testosterone activates anabolic pathways via IGF1-mediated stimulation of the Akt-mTOR pathway, activation of satellite cells and inhibition of myostatin activity (partly via follistatin). In the opposing process, myostatin inhibits FoxO-mediated proteasomal and lysosomal catabolic pathways and satellite cell activation/proliferation. Testosterone also delays the transition from myoblast to myotube, maintaining the myoblasts in their proliferative state for longer. Compiled based on Basaria & Bhasin (2012), Basualto-Alarcon et al. (2013),

Braga et al. (2012), De Naeyer et al. (2014), Deane et al. (2013), Dubois et al. (2012, 2014), Haren et al. (2011), Herbst & Bhasin (2004), Ibebunjo, et al. (2011), Jasuja et al. (2014), Jones et al. (2010), Lee et al. (2011), Mendler et al. (2007), Rana et al. (2014b), Rodriguez et al. (2011, 2014), Serra et al. (2013a), Sinha-Hikim et al. (2006), Svensson, et al. (2010) and White et al. (2013). ActRIIB, activin type II receptor type B; mTOR, mammalian target of rapamycin; FoxO, forkhead box O; AMPK, 5'AMP-activated protein kinase; MAFbx, muscle atrophy F-box; MuRF1, muscle ring finger 1; MRF, myogenic regulatory factor; CDK, cyclin-dependent kinase; Pax, paired box genes.

understood (Dubois et al. 2012). Much of the recent understanding of androgen-mediated skeletal muscle signaling has been derived from identification of testosterone-regulated genes and their differential expression using microarray technology (Table 1). Current evidence suggests that testosterone may exert its anabolic effect in a number of ways.

Activation of satellite cells

First, testosterone may increase muscle cell mass through the activation of satellite cells via the AR (Serra et al. 2013b, Dubois et al. 2014). The exact effects of testosterone on proliferation and differentiation of satellite cells (and their active form, myoblasts) are not fully elucidated; however,

Table 1 Comparison of recent relevant studies using microarray in androgen deprivation models

Authors	Androgen deprivation model	Groups	Main outcome/aims	Relevant findings Unless otherwise noted, all findings described have a <i>P</i> value <0.05
Liu et al. (2013)	Muscle tissue from four elderly men, sample taken from biceps brachii	Group 1: Four older men Group 2: Four older women Group 3: Seven younger men Group 4: Seven younger women	Identification of transcriptional differences between older men and women and sex-specific aging differences	<p>Provided evidence for the presence of sex differences in the aging process of skeletal muscle</p> <p>Older men vs older women:</p> <ul style="list-style-type: none"> ↑ expression of genes involved with mitochondrial structure and function (no difference observed when comparing younger men with younger women) ↓ expression of genes involved with extracellular matrix remodelling and immune function <p>Older men vs younger men</p> <ul style="list-style-type: none"> Some differences in transcript levels; functional significance of these genes uncertain <p>Regulation of genes differed between the different models used</p> <ul style="list-style-type: none"> ↓ expression of myogenin in group 1 vs group 2 ↑ myogenin expression in ARKO vs WT mice ↑ expression of genes regulating the transition from proliferation to differentiation in the ARKO model ↓ <i>c-myc</i>, a promoter of proliferator, in testosterone-treated cell culture, WT mice and orchiectomized mice treated with testosterone ↓ ubiquitin ligase expression in testosterone-treated cell culture, no difference in orchiectomized or ARKO models <p>Group 1 vs Group 2:</p> <ul style="list-style-type: none"> ↓ total-limb maximal grip strength by 7% Altered fiber-type distribution in soleus (↑ type I, ↓ type IIA), no change in distribution in EDL ↓ weight of levator ani (−52%); no effect on limb skeletal muscle mass ↓ expression of myostatin (sixfold, confirmed by quantitative real-time PCR) <p>Found a discrepancy in the androgenic modulation of myostatin mRNA (↓ in levator ani/bulbocavernosus (LA/BC), ↑ in extensor digitorum longus)</p> <ul style="list-style-type: none"> ↑ expression of ubiquitin ligases in LA/BC following orchiectomy ($P<0.001$), including <i>MAFbx/Atrogin-1</i> and <i>Murfi1</i>, demonstrating increased FoxO activity <p>These changes were variably reversed with testosterone administration; no reversal with E₂ administration</p>
Rana et al. (2014b)	Castrated male mice and ARKO male mice	<p>Group 1: Orchiectomized adult male mice treated with testosterone (supra-physiological dose)</p> <p>Group 2: Orchiectomized adult male mice treated with placebo</p> <p>Group 3: Adult ARKO male mice</p> <p>Group 4: Adult WT mice</p> <p>Group 5: Human skeletal muscle cell culture treated with dihydrotestosterone (DHT)</p>	Identification of AR-regulated genes in skeletal muscle	<p>Examination of the consequences of AR loss in satellite cells on muscle mass, fiber type, limb muscle grip strength and gene expression</p>
Dubois et al. (2014)	Satellite cell-specific knockout (satARKO) male mice	Group 1: satARKO male mice Group 2: control male mice	Examination of the effect of androgen deprivation, T and E ₂ administration on muscle mass and expression of <i>MAFbx/Atrogin-1</i> , <i>Murfi1</i> and myostatin	<p>Investigation of the effect of androgen deprivation, T and E₂ administration on muscle mass and expression of <i>MAFbx/Atrogin-1</i>, <i>Murfi1</i> and myostatin</p>
De Naeyer et al. (2014)	Castrated male mice	<p>Group 1: Orchiectomized adult male mice treated with testosterone</p> <p>Group 2: Orchiectomized adult male mice treated with estradiol (E₂)</p> <p>Group 3: Orchiectomized adult male mice treated with vehicle</p> <p>Group 4: Sham operated male mice</p>	Investigation of the effect of androgen deprivation, T and E ₂ administration on muscle mass and expression of <i>MAFbx/Atrogin-1</i> , <i>Murfi1</i> and myostatin	<p>Investigation of the effect of androgen deprivation, T and E₂ administration on muscle mass and expression of <i>MAFbx/Atrogin-1</i>, <i>Murfi1</i> and myostatin</p>

Table 1 Continued

Authors	Androgen deprivation model	Groups	Main outcome/aims	Relevant findings Unless otherwise noted, all findings described have a <i>P</i> value <0.05
Jasuja et al. (2014)	Castrated male mice	Group 1: Castrated adult male mice treated with recombinant follistatin (rFst) Group 2: Castrated adult male mice treated with testosterone Group 3: Castrated adult male mice treated with vehicle Group 4: Sham operated male mice treated with vehicle	Identification of genes differentially regulated by testosterone and rFst in prostate and levator ani	Identified 852 androgen-sensitive genes and 778 follistatin-sensitive genes. 391 genes responsive to both androgens and follistatin ↑ muscle mass with rFst administration; no effect on prostate mass
Haren et al. (2011)	Castrated male mice	Group 1: Castrated adult male mice treated with testosterone Group 2: Castrated adult male mice treated with placebo Group 3: Sham operated male mice treated with placebo	Identification of early transcriptional changes following castration and testosterone replacement (prior to changes in gastrocnemius mass)	↑ expression of 68 genes with testosterone administration ↓ expression of 91 genes with testosterone administration ↑ ubiquitin ligase (<i>Maifbx/Atrogin-1</i>) mRNA levels with testosterone administration, in contrast to previous studies Testosterone modulated a number of genes involved in nutrient processing, suggesting testosterone may increase the availability of substrates for protein synthesis
Ibebunjo et al. (2011)	Castrated male mice	Group 1: Orchiectomized adult male mice treated with testosterone Group 2: Orchiectomized adult male mice treated with vehicle Each group was further subdivided into sedentary or access to running wheel and duration of testosterone treatment	Examination of the effects of orchiectomy and testosterone replacement on voluntary running, skeletal muscle mass and histology and gene expression	↑ expression of ubiquitin ligases <i>Murf1</i> and <i>Maifbx</i> with orchiectomy, reciprocal, ↓ expression with testosterone administration ($P < 0.001$) ↓ expression of <i>Igf1</i> with orchiectomy ($P < 0.001$), variable ↑ with testosterone administration. IGF1 signaling activity showed same pattern (western blot analysis) ↓ expression of myostatin with orchiectomy ($P < 0.001$), reciprocal ↑ in some groups with testosterone administration ↓ expression of <i>Igf1</i> with E_2 but serum IGF1 levels ↑
Svensson et al. (2010)	Castrated male mice	Group 1: Orchiectomized male mice treated with dihydrotestosterone (DHT) Group 2: Orchiectomized male mice treated with estradiol (E_2) Group 3: Orchiectomized male mice treated with vehicle (control)	Examination of changes in gene expression and muscle mass following AR and estrogen-receptor stimulation	DHT, and E_2 regulated different sets of genes (except 13); of the 13 common genes ↑ <i>Fbxo32</i> (<i>Atrogin-1/Maifbx</i>) expression with both testosterone ($P < 0.01$) and E_2 ($P < 0.05$) administration
MacLean et al. (2008)	Castrated male mice and ARKO male mice	Group 1: ARKO male mice Group 2: WT male mice Group 3: ARKO female mice Group 4: WT female mice	Determination of consequences of AR knockout in female and male mice	↓ muscle mass in ARKO male ($P < 0.001$), not ARKO female No change in <i>Igf1</i> expression, IGF1 serum levels or expression of genes encoding IGF1-binding proteins or the IGF1 receptor in ARKO males No change in expression of ubiquitin ligases

the data generally suggests that androgens increase satellite cell numbers and are associated with increased satellite cell activation (Sinha-Hikim *et al.* 2003, Oner *et al.* 2008, Serra *et al.* 2013b, Sipila *et al.* 2013, Dubois *et al.* 2014). This is complicated by the suggestion that many factors (such as nitric oxide, follistatin, interleukin-6 and notch signaling (see below)) may contribute to satellite cell activation (Dubois *et al.* 2012). Likewise, increases in the satellite cell number may be due to a combination of multiple causes, such as increased replication, inhibition of apoptosis and increased differentiation of multipotent stem cells into the myogenic lineage (Bhasin *et al.* 2003, Sinha-Hikim *et al.* 2006). This concept of testosterone as a modulator of lineage determination has been demonstrated experimentally, illustrating that androgen activation of mesenchymal multipotent stem cells promotes myogenic differentiation but inhibits adipogenesis, thereby affecting both fat and muscle lineages (Bhasin *et al.* 2003, 2006, Singh *et al.* 2003). Therefore, in androgen-deficient states, the opposite may occur, leading to sarcopenic obesity. There is also some evidence from animal models suggesting that the activation of the AR represses translation of genes responsible for the transition from myoblast proliferation to differentiation, maintaining the myoblasts in their proliferative state for a longer duration (Lee *et al.* 2011, Rana *et al.* 2014b).

Stimulation of the GH/IGF1 axis

Second, androgen-induced stimulation of the GH/IGF1 axis leads to the activation of the anabolic Akt-mTOR pathway (Basaria & Bhasin 2012, Dubois *et al.* 2012, Deane *et al.* 2013, Purves-Smith *et al.* 2014). Studies regarding the effect of testosterone administration on IGF1 levels and the impact of this on muscle mass are not fully concordant, but it is generally agreed that IGF1 is important for mediating the anabolic effects of testosterone, primarily via promoting proliferation and differentiation of myoblasts, as well as influencing satellite cell activation and/or number (Chen *et al.* 2005, Sinha-Hikim *et al.* 2006, MacLean *et al.* 2008, Svensson *et al.* 2010, Ibebunjo *et al.* 2011, Serra *et al.* 2013b, White *et al.* 2013). GH/IGF1 may also modulate myostatin expression, but data is limited, and it is inherently difficult to determine whether any inhibitory effects of GH are due to GH administration directly or as a secondary effect of increased muscle mass (Liu *et al.* 2003, Rodriguez *et al.* 2014). One potential mechanism suggested for the loss of muscle mass following ADT is that decreased IGF1 levels may reduce Akt/mTOR-stimulated

anabolic activity (Jones *et al.* 2010). In animal models, castration-induced androgen deficiency has been associated with suppressed Akt/mTOR activation and higher levels of catabolic forkhead box (FoxO) mRNA, which would lead to decreased muscle growth (Bhasin *et al.* 2006, Serra *et al.* 2013a,b, White *et al.* 2013). However, reduced IGF1 levels and/or mRNA expression has not been consistently demonstrated in mouse models, despite the increased IGF1 serum levels generally seen following testosterone administration (MacLean *et al.* 2008, Jones *et al.* 2010, Svensson *et al.* 2010, Ibebunjo *et al.* 2011, White *et al.* 2013). A number of possible interactions have also been identified, such as the removal of downregulation of 5'AMP-activated protein kinase (AMPK) by testosterone, the activation of which leads to muscle atrophy, therefore potentially altering the balance between AMPK and mTOR resulting in net muscle loss (Serra *et al.* 2013a).

Repression of myostatin activity

Third, testosterone may exert its anabolic effect via repression of myostatin expression and/or activity, thereby inhibiting muscle breakdown, as myostatin acts as a repressor of translation initiation (Basaria & Bhasin 2012, Rodriguez *et al.* 2014). There is substantial evidence for the importance of myostatin as a negative regulator of muscle growth, and there are many downstream effects of myostatin activation or inhibition (Basaria & Bhasin 2012, Dubois *et al.* 2012, Rodriguez *et al.* 2014). Androgens reduce myostatin expression; however, the extent to which this occurs via direct inhibition by testosterone or indirectly via another mediator is uncertain, and not all studies concur (Mendler *et al.* 2007, Ibebunjo *et al.* 2011, De Naeyer *et al.* 2014, Dubois *et al.* 2014, Grossmann 2014, Rodriguez *et al.* 2014). Despite this uncertainty, the removal of inhibition of myostatin appears to be an important factor in androgen-deficient muscle wasting (Ibebunjo *et al.* 2011, Basaria & Bhasin 2012, De Naeyer *et al.* 2014, Dubois *et al.* 2014, Grossmann 2014, Padhi *et al.* 2014, Rodriguez *et al.* 2014). Several studies have shown that myostatin decreases the activation of the anabolic Akt-mTOR pathway and signals via the FoxO transcription factors to induce skeletal muscle atrophy, and androgen deprivation has been demonstrated to induce the expression of these FoxO targets (Jones *et al.* 2010, Serra *et al.* 2013a, White *et al.* 2013, Rodriguez *et al.* 2014). The extent to which this occurs via proteosomal vs lysosomal pathways requires further investigation; however, the autophagy-lysosomal system is generally thought

to be less important (Ciciliot *et al.* 2013). The role of myostatin as a key modulator of androgen-deficient muscle wasting has been given further weight in a recent phase I trial of a myostatin inhibitor in men receiving ADT for non-metastatic prostate cancer (Padhi *et al.* 2014). The myostatin inhibitor increased muscle mass by 1.5% ($P=0.008$) and decreased fat by 1.7% ($P=0.021$), as measured by dual energy X-ray absorptiometry (DEXA) (Padhi *et al.* 2014). However, there was no significant effect on muscle strength, measured by the maximum weight lifted in one repetition using a knee extension machine (1-RM), or physical function as assessed by the short physical performance battery (SPPB) (Padhi *et al.* 2014). It is possible that this is a result of the duration of treatment (28 days) or the choice of method for measuring physical function (Padhi *et al.* 2014). It is not clear whether the SPPB is a sensitive parameter to measure physical function, primarily because the specific deficit seen in men taking ADT is yet to be defined (Cheung *et al.* 2014). There were no significant changes in metabolic parameters such as fasting glucose, insulin or lipid levels, which is not unexpected given that these parameters are unlikely to change significantly in 28 days. Follistatin, an autocrine glycoprotein, has also been implicated in mediating the promyogenic effects of testosterone by the inhibition of myostatin signaling in animal models and cell cultures and is also the subject of clinical trials in treating sarcopenia (Basaria & Bhasin 2012, Braga *et al.* 2012, Jasuja *et al.* 2014, Rodriguez *et al.* 2014). This potential therapeutic applicability, combined with the complexity of androgen-deficient signaling disruption, underscores the need to develop a more in-depth understanding of this research area.

Enhancement of notch signaling

Testosterone may also act via the enhancement or activation of notch signaling pathways. This is a newer concept, and the exact involvement of testosterone is not yet clear (Sinha-Hikim *et al.* 2006, Dubois *et al.* 2012). Notch signaling is essential for satellite cell proliferation and myogenic progression and may be part of the mechanism of satellite cell activation via testosterone (Sinha-Hikim *et al.* 2006). Notch expression has been demonstrated to increase after testosterone administration; however, it is unclear whether this is a direct effect or the result of partial regulation by other elements of the skeletal muscle metabolism cascade (Sinha-Hikim *et al.* 2006, Brown *et al.* 2009, Dubois *et al.* 2012).

Non-genomic mechanisms

A number of non-genomic factors have also been suggested, such as the modulation of kinase activity and increase of calcium uptake in muscle, but the exact mechanisms of these effects and their relative contributions to overall muscle growth is yet to be determined (Bhasin *et al.* 2006, Dubois *et al.* 2012). As testosterone is aromatized to estradiol in target tissues, it is difficult to determine the extent to which the anabolic actions of testosterone are due to AR or estrogen receptor activity (Bhasin *et al.* 2006, Svensson *et al.* 2010, Finkelstein *et al.* 2013, Dubois *et al.* 2014).

Clinical context

Ultimately, understanding these pathways should guide the rational development of novel therapeutic targets designed to prevent or even reverse adverse changes in muscle mass and muscle function. At present, exercise is the only available therapy for sarcopenia, and a randomized controlled trial (RCT) has demonstrated that supervised exercise can mitigate adverse body composition changes associated with ADT over 3 months; however, the functional benefits were less consistent (Cormie *et al.* 2014). Although exercise appropriate to a patient's functional status has multiple health benefits and should be recommended routinely, engaging patients to perform exercise and ensuring safety and cost-effectiveness are challenging. Exercise and muscle contraction can stimulate myokine release, such as follistatin or Akt signaling (Hansen *et al.* 2011). Further research should focus on not only delineating the signaling pathways regulated by exercise on muscle but also determining which modes of exercise and subsequent signaling effects are able to improve function in addition to mass.

Novel pharmaceuticals to improve muscle function, for example, the anti-myostatin peptibody AMG 745, are being tested in early phase clinical trials (Padhi *et al.* 2014). However, no conclusive evidence regarding their efficacy and safety is available, and comparisons to exercise programs have not yet been performed. Selective androgen receptor modulators are also an emerging class of anabolic agents that are selective in their effect on muscle and bone. There is no data for use in prostate cancer in humans; however, animal models are encouraging. The manipulation of these and other testosterone-regulated targets such as activin receptor type IIB inhibitors or a variety of genetic approaches is an active area of research

that may pave the way to new treatments of sarcopenia and dynapenia (Cheung *et al.* 2014).

Conclusions

Although the mechanisms by which ADT-induced sarcopenia occurs at a cellular, protein and gene level are not fully understood, some inferences can be made from the more extensively studied area of testosterone-influenced anabolic signaling pathways. Given that muscle is one of the most androgen-responsive organs, it is not surprising that the actions of testosterone on muscle are complex and involve multiple cellular pathways. Indeed, the challenge lies in the dissection of the relative biological importance of these actions in governing functional muscle performance, which may well vary in different clinical scenarios. At the same time, mechanistic studies can uncover multiple possible targets that have the potential to be exploited therapeutically. Be it in men with prostate cancer receiving ADT or in the aging individual, an effective therapeutic strategy is likely to require a combination of targeted pharmaceutical agents with a tailored exercise program. Clearly, further basic research is essential to elucidate mechanisms underlying testosterone-regulated myokines and genes, making this a fertile area for future research.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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