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To cite this article: Melinda Sheffield-Moore (2000) Androgens and the control of skeletal muscle protein synthesis, Annals of Medicine, 32:3, 181-186, DOI: 10.3109/07853890008998825

To link to this article: https://doi.org/10.3109/07853890008998825

Published online: 08 Jul 2009.

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Androgens and the control of skeletal muscle protein synthesis

Melinda Sheffield-Moore

Athletes have long supported the concept that anabolic steroids increase skeletal muscle mass. However, it was only recently that both testosterone and its synthetic analogue, oxandrolone, were proven capable of inducing myotrophic effects in postabsorptive human skeletal muscle. These findings have provided the physiological evidence that anabolic steroids deserve attention in the clinical arena as a pharmacological intervention against losses in lean body mass associated with age, disease, trauma and burn injury. However, we are lacking in vivo molecular evidence that would directly or indirectly link androgens and the androgen receptor with increases in skeletal muscle mass. Clearly, a need exists to link in vivo and in vitro studies from both the physiological and molecular arena as they relate to androgens and the control and regulation of skeletal muscle mass. In this brief review, newly discovered information and emerging theories relating to the direct, indirect, priming and antiglucocorticoid action of androgens on skeletal muscle will be presented.

Key words: androgens; androgen receptors; protein synthesis; skeletal muscle mass.


Introduction

In the last decade, the body of knowledge surrounding the physiological and molecular mechanisms responsible for inducing myotrophic effects in skeletal muscle in response to anabolic-androgenic steroids has increased at a modest pace. The ability to measure skeletal muscle protein synthesis and breakdown in humans by using stable isotopes and muscle biopsies (1, 2) and the cloning of the human androgen receptor cDNA (3-6), have greatly enhanced our limited understanding of the tissue-specific actions of testosterone and other steroid agents. However, despite all we have learned, the mechanisms by which androgens directly or indirectly mediate increases in human skeletal muscle tissue remain poorly understood. Therefore, the aim of this brief review is to merge findings from physiological and molecular-based studies of androgens and to outline the potential mechanisms responsible for regulating androgen-induced increases in human skeletal muscle protein.

Physiology of androgen action

Androgens are responsible for the induction of the male phenotype, from sexual differentiation in utero, to the development of secondary sex characteristics in puberty and throughout adulthood (maintaining sexual function and fertility). Moreover, androgens are biologically diverse targeting both reproductive and nonreproductive tissues, such as the brain, liver, kidney, prostate, bone and skeletal muscle tissue. The diversity of androgens is largely the result of the ability of testosterone to act as a circulating precursor or prohormone for the formation of two active metab-
olites, dihydrotestosterone and oestradiol. Specifically, testosterone can undergo irreversible reduction to the \( \Delta^{4} \)-3-keto configuration which can be further metabolized to 17-ketosteroids and polar derivatives. On the other hand, circulating androgens of testosterone to dihydrotestosterone and oestradiol. Thus, testosterone, oestrogen and active metabolites, dihydrotestosterone and oestradiol are poorly understood. In addition to the obvious role that androgens play in male development and in the maintenance of the male phenotype, androgenic hormones also display potent protein anabolic effects in human skeletal muscle.

In normal eugonadal men, mixed muscle protein synthesis is increased following administration of testosterone (11, 12) and oxandrolone (13), a synthetic analogue of testosterone. In addition, testosterone replacement therapy enhances skeletal muscle mass (14–16) and increases protein synthesis in hypogonadal men (14). Likewise, hypogonadal elderly men receiving testosterone replacement therapy demonstrate increases in protein synthesis (18), lean body mass (17) and muscular strength (18). Further, in hypogonadal men with AIDS wasting syndrome, losses in lean body mass are highly correlated with androgen levels (19). These primarily physiology-based clinical studies clearly demonstrate that the steroid hormone testosterone, and its synthetic analogue oxandrolone, have the potential to increase human skeletal muscle mass by increasing protein synthesis.

**Direct mechanisms of androgen receptor activation and function**

As recently as 5 years ago, there was considerable speculation as to whether anabolic–androgenic steroids act directly on skeletal muscle by binding to androgen receptors and thereby activating protein synthesis via the enhancement of transcription factors, now more commonly referred to as the steroid hormone receptor superfamily. Current evidence exists to support the contention that androgens act in their target cells via both a direct and an indirect interaction with the androgen receptor, resulting in the regulation of gene expression.

In concert with the diversity of androgens, the androgen receptor displays considerable biological diversity and mediates a broad range of developmental and homeostatic events in the male phenotype. Despite this diversity, androgens induce their specific response via a single receptor protein, the androgen receptor, which is encoded on the X chromosome (Fig 1). Only one androgen receptor cDNA has been identified and cloned (3–6) and androgen receptors (20) and androgen receptor mRNA (21) have been detected in human skeletal muscle. Two tissue-specific ligands mediate the androgen receptor, testosterone and its \( \Delta^{4} \)-reduced metabolite, \( \Delta^{5} \)-dihydrotestosterone. Because the activity of \( \Delta^{5} \)-reductase is minimal in skeletal muscle, it appears that testosterone is the key hormone for androgen action in skeletal muscle.

While we know that an accumulation of DNA is essential for muscle growth, the mechanisms of androgen-induced DNA accretion in skeletal muscle are unclear. It has been suggested that the tissue-specific actions of testosterone and dihydrotestosterone evoke a ligand-specific recruitment of transcription intermediary factors (TIFs) for the androgen receptor (23), although no evidence exists to support this contention. However, current evidence obtained with exercising rats indicates that the accretion of skeletal muscle may be dependent on an increased number of androgen receptors (24). Inoue and co-workers (24) examined the physiological importance of the increase in androgen receptors on exercise-induced muscle hypertrophy. They determined that the androgen pathway had a significant effect on exercise-induced muscle hypertrophy and found the hypertrophy to be associated with an increased number of androgen receptors in the exercised muscle (24).

More recently, we found androgen receptor mRNA concentrations to be increased in skeletal muscle following short-term exposure (5 days) to a synthetic analogue of testosterone, oxandrolone (13). In addition, skeletal muscle protein synthesis increased following administration of oxandrolone in young healthy men, despite a significant suppression of endogenous testosterone (13). Thus, oxandrolone, similar to testosterone, appears to mediate the observed increases in muscle protein synthesis via direct interaction with the activated androgen receptor.

**Figure 1.** Schematic diagram of the normal androgen receptor.
**Indirect mechanisms of androgen action**

Although the indirect mechanisms of androgen action are undoubtedly important in the induction and modulation of androgen action they far exceed the scope of this review. However, it is worth noting what is known about the indirect effects of androgen action. Unlike the direct effects of androgens, the indirect effects of androgens do not depend upon a direct interaction with the androgen receptor. Instead, such effects may induce or modulate the activity of secondary transcription factors that influence the expression of indirectly regulated genes in the same target cell (25). On the other hand, they may modulate gene expression by producing or controlling the activity of autocrine or paracrine mediators via membrane receptors. Finally, they are capable of mediating the potential effects androgens have on distant tissues by reversibly or irreversibly changing the secretion of other hormones (25).

A recent study found that testosterone is capable of indirectly mediating increases in muscle mass by increasing hepatically-derived insulin-like growth factor (IGF-I) in sheep (26). Further, it has been suggested that prior cellular exposure to androgens may somehow prime cells for the action of secondary agents such as IGF-I. Recent evidence lends support to the complementary role of androgens, androgen receptors and IGF-I. IGF-I mRNA has been shown to increase in skeletal muscle of elderly men treated with 4 weeks of replacement doses of testosterone enanthate (18). Conversely, marked decreases in IGF-I mRNA concentrations occur following induction of severe androgen deficiency in normal young men (27). Within skeletal muscle tissue, androgens appear to be necessary for local IGF-I production, independent of growth hormone (GH) production and systemic IGF-I concentrations (27). Recent evidence from these same young men indicates that androgen receptors are significantly decreased in response to severe hypogonadism. Thus, androgens probably work directly through the androgen receptor to exert their effects on protein metabolism acting as transcriptional enhancers thereby influencing the expression of specific genes.

Similarly, it is plausible that androgens have a temporal effect on skeletal muscle and require a minimum, as yet unknown, period of time to mediate a protein synthetic effect via the enhancement of transcription. Pretreatment of porcine satellite cells with testosterone for 24 h up-regulated androgen receptors, but did not alter the responsiveness of these cells to IGF-I or other growth factors (29). Further, a stimulation of protein synthesis has been reported in human skeletal muscle following a 3–6 h arterial infusion of IGF-I (30). Using stable isotopic techniques, our laboratory previously measured skeletal muscle protein synthesis in normal young men in the basal period and then following an acute 5-h infusion of testosterone. No change in muscle protein synthesis occurred with this brief androgen exposure indicating that translation of proteins was not affected (31). Conversely, 5 days of oxandrolone was sufficient to increase skeletal muscle protein synthesis and androgen receptor mRNA concentrations in normal young males (13), indicating that 5 days of androgen exposure is a sufficient time to enhance transcription and expression of target genes in muscle. Clearly, there is a need for more in vivo time course studies examining the protein synthetic response of testosterone and other steroid hormones.

There is some indirect evidence that the anabolic effects of testosterone and other androgenic–anabolic agents on skeletal muscle may be mediated through an antiguocorticoid action (32). Androgenic–anabolic steroids are thought to be capable of displacing glucocorticoids bound to the glucocorticoid receptor. This seems plausible as a large degree of homology exists in the DNA-binding and ligand-binding domains of the androgen receptor with its other members of the steroid hormone receptor superfamily (eg, receptors for glucocorticoids, oestradiol, progesterone and mineralocorticoids) (3, 33–36). In vitro work suggests that testosterone has a very high binding affinity for the glucocorticoid receptor (37) and that testosterone exerts anabolic activity by acting as an antagonist to endogenous glucocorticoids (38). Still others, such as Hickson and co-workers, propose that glucocorticoid activity at the gene level is inhibited via androgen interference with the DNA binding region known as the glucocorticoid response element (32).

Recent evidence lends credence to this theory as antagonists of glucocorticoid action are capable of preventing orchidectomy-induced muscle atrophy (39). Unfortunately, in our 5-day oxandrolone study in normal males (13), we saw no definitive response with the glucocorticoid receptor as three subjects showed an increase in glucocorticoid mRNA concentration, one subject showed a decrease and one remained unchanged (M Sheffield-Moore, unpublished observations, 1999). However, following oxandrolone administration and an overnight fast, protein synthesis was significantly increased while protein breakdown remained unchanged. Normally, protein breakdown would be greatly increased following an overnight fast in normal subjects. This serves as indirect evidence that oxandrolone may act as an antagonist to normal glucocorticoid action in postabsorptive skeletal muscle by binding to the glucocorticoid receptor, thereby preventing its normal protein catabolic action.

We have additional indirect evidence of the possible antagonist role of androgens on glucocorticoid action from a study of adult males with severe burn injury. Normally, severe burns cause dramatic losses in lean body mass such that protein breakdown far exceeds

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protein synthesis. Also, burn injury suppresses production of endogenous testosterone further exacerbating protein catabolism. This decrease in endogenous testosterone can last for several months postburn (40). To further complicate matters, endogenous production of cortisol is greatly increased, persists for quite some time, and is related to the severity of the injury (41). Therefore, we sought to assess the effects of testosterone normalization on skeletal muscle in males with severe burn injury. Muscle protein synthesis and breakdown were evaluated by stable isotope infusion and arteriovenous model with muscle biopsy at baseline and after 2 weeks of testosterone enanthate (200 mg injected once a week for 2 weeks). Following the first injection of testosterone, blood concentrations of testosterone increased to the low normal range in all subjects. By 2 weeks of supplementation, testosterone levels reached upper normal levels. Interestingly, we found no change in protein synthesis, but protein breakdown decreased nearly twofold after testosterone normalization. Therefore, overall net protein balance was significantly improved (42). These data, along with our previous study using oxandrolone in normal subjects (13), support the concept that testosterone may mediate anabolism in skeletal muscle through its role as a glucocorticoid antagonist. Nevertheless, in each of these cases, it is clear that the indirect effects of androgens are critical in the overall response that androgens have on genes, cells and tissues. Unfortunately, the nature by which androgens indirectly effect skeletal muscle tissue remains unclear.

Nonandrogen mechanisms controlling skeletal muscle mass

Recent discoveries relating to the nonandrogenic control and regulation of skeletal muscle mass have been elucidated and deserve mention in this review. While other nonandrogen compounds, such as insulin and GH, have anabolic effects on skeletal muscle, this discussion will only focus on newly discovered mechanisms and by no means is intended to be exhaustive.

One exceptionally important discovery is the myostatin gene. Myostatin, a member of the transforming growth factor (TGF)-β superfamily, has recently been shown to be a key genetic determinant and negative regulator of skeletal muscle growth (43, 44). Mice (45) and cattle (43, 44) have both displayed what has been termed ‘double-muscling’ as a result of mutations in the myostatin-coding sequence (43). Interestingly, it appears that the function of myostatin has been highly conserved among vertebrates as there is great similarity in the phenotypes of double-muscled cattle and myostatin null mice, suggesting that myostatin performs the same biological function in these two species (43). Not surprisingly, HIV-infected men suffering from disease-induced losses in skeletal muscle mass have recently been shown to have an increased expression of myostatin-immunoreactive protein as compared with normal controls (46). More importantly, myostatin-immunoreactive protein is not only uniquely expressed in human skeletal muscle, but is also secreted into plasma suggesting that the presence of receptors might exist in the muscle and other locations involved in the regulation of skeletal muscle mass (46). Therefore, the ability to inhibit myostatin expression in humans with age, cancer, HIV or trauma-related losses in skeletal muscle mass may have profound clinical implications.

For some time, researchers have attempted to uncover the molecular signalling pathways that link increases in skeletal muscle usage to changes in skeletal muscle size. Recently, a breakthrough finding has implicated a calcium-regulated phosphatase, calcineurin, in the signalling of some forms of cardiomyopathic growth (47, 48). More recently, calcineurin has been shown to either directly or indirectly influence the accumulation of skeletal muscle contractile proteins under conditions of increased overload or activation (49). The findings by Barton-Davis and co-workers (50) that virally delivered IGF-1 genes induce local skeletal muscle hypertrophy, attenuate age-related skeletal muscle atrophy and restore muscle mass and strength in mice has led to additional breakthrough findings that IGF-I and calcineurin are capable of inducing skeletal muscle hypertrophy (51). These findings provide considerable insight to the potential mechanisms behind muscle wasting and functional losses associated with neuromuscular diseases, aging and cancer cachexia.

Finally, a novel concept of developing molecules capable of acting as ligands with a high degree of tissue selectivity has recently been proposed to replace traditional androgen therapies in men and women. These compounds have been termed selective oestrogen receptor modulators (SERMs) and selective androgen receptor modulators (SARMs) (52). The recent development and marketing of SERMs has provided preliminary evidence that molecules can be developed exhibiting a great degree of tissue selectivity (targeting the oestrogen receptor) and, at the same time, remain safe and efficacious at selectively activating transcriptional receptors (53, 54). While the potential for these compounds has yet to be fully realized, the ability to target specific tissues and disorders toward a positive therapeutic outcome has clear advantages over traditional hormone replacement therapies.

Conclusion

Much of what is known about how androgens induce increases in skeletal muscle mass has been discovered

only recently. Unfortunately, this review serves to remind us that the mechanisms linking the physiological and molecular actions of anabolic steroids and the regulation of skeletal muscle mass are poorly understood, and will require further scientific analysis to uncover both the direct and indirect nature of these processes. Nevertheless, emerging evidence from both physiological and molecular-based clinical studies in populations suffering from androgen insensitivity syndromes and protein catabolic events associated with disease and trauma will continue to further define our understanding as to how androgens and non-androgens control skeletal muscle mass.

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