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Androgen-mediated regulation of skeletal muscle protein balance

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ABSTRACT

Androgens significantly alter muscle mass in part by shifting protein balance in favor of net protein accretion. During various atrophic conditions, the clinical impact of decreased production or bioavailability of androgens (termed hypogonadism) is important as a loss of muscle mass is intimately linked with survival outcome. While androgen replacement therapy increases muscle mass in part by restoring protein balance, this is not a comprehensive treatment option due to potential side effects. Therefore, an understanding of the mechanisms by which androgens alter protein balance is needed for the development of androgen-independent therapies. While the data in humans suggest androgens alter protein balance (both synthesis and breakdown) in the fasted metabolic state, a predominant molecular mechanism(s) behind this observation is still lacking. This failure is likely due in part to inconsistent experimental design between studies including failure to control nutrient/feeding status, the method of altering androgens, and the model systems utilized.

Contents

Abbreviations: mTORC1, mechanistic target of rapamycin in complex 1; AR, androgen receptor; DHT, 5α-dihydrotestosterone; TE, testosterone enanthate; TC, testosterone cypionate; ARE, androgen response element; p70S6K1, 70 kD ribosomal protein S6 kinase 1; 4E-BP1, eukaryotic initiation factor 4E binding protein 1; TSC2, tuberous sclerosis 2/tuberin; PRAS40, proline-rich Akt substrate of 40 kD; AMPK, 5′ AMP-activated protein kinase; REDD1, regulated in development and DNA damage 1; UPS, ubiquitin proteasome system; BNIP3, BCL2/adenovirus E1B 19kDa interacting protein 3; MuRF-1, muscle RING-finger protein-1; MAFbx/atrogin-1, muscle Aatrophy F-box; FoxO, forkhead box; IGF-1BP-4, IGF-1 binding protein 4; PI3K, phosphatidylinositol-3 kinase; ERK1/2, extracellular signal-regulated kinase 1/2; SNAT2, sodium-coupled neutral amino acid transporter 2; LAT2, L-type amino acid transporter 2; rps6, ribosomal protein s6; EDL, extensor digitorum longus; TA, tibialis anterior; Raptor, regulated associated protein of mTOR; c-myc, v-myc avian myelocytomatosis viral oncogene homolog; Nop56, nuclear protein 56; Bop1, block of proliferation1; Ncl, nucleolin; TGFβ, transforming growth factor beta; GDF11, growth differentiation factor 11; Tfeb, transcription factor EB; LC3, microtubule-associated protein 1A/1B-light chain 3; ULK1, uncoordinated like kinase 1; PCNA, proliferating cell nuclear antigen; DHEA, Dehydroepiandrosterone; MUSA1, Muscle ubiquitin ligase of SCF complex in atrophy 1.

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1. Introduction

The importance of maintaining skeletal muscle mass during various catabolic conditions is becoming increasingly recognized since muscle wasting into older age is predictive of an unfavorable survival outcome (Martin et al., 2013). In males, reduced production or bioavailability of androgens, termed hypogonadism, directly contributes to muscle atrophy since androgens play a major role in the maintenance or restoration of muscle mass (Ferrando et al., 2003; Steiner et al., 2017, White et al., 2013a,b; White et al., 2013a,b; Atkinson et al., 2010). While a therapy such as resistance exercise is effective at increasing muscle mass during hypogonadal conditions (Sullivan et al., 2005), there is also evidence that resistance exercise cannot increase mass to the same absolute value achieved by those with circulating androgen levels in the physiological range (Kvorning et al., 2006). This highlights the important physiological role of androgens, in conjunction with other factors such as physical activity, in the overall maintenance of muscle mass.

Androgen-mediated changes in muscle mass are due in part to alterations in muscle protein balance with hypogonadism shifting this balance in favor of net protein breakdown (Ferrando et al., 2003; Ferrando et al., 1998; Sheffield-Moore et al., 1999). Several studies have examined the molecular factors implicated in androgen-mediated changes in muscle size and protein metabolism (i.e. (Hughes et al., 2012); however, numerous experimental inconsistencies preclude a definitive conclusion from being made about the predominant factors/pathways contributing to this change in protein balance. This is important because mimicking the effects of androgens pharmacologically to increase muscle mass is required for those individuals in which androgen replacement is not a treatment option due to potentially negative side effects (Atkinson et al., 2010; Bassil et al., 2009). For example, androgens may augment the growth of a cancer tumor, making androgen replacement a non-viable option for those with established cancer tumors and suffering from cancer cachexia (Huggins and Hodges, 2002; Amos-Landgraf et al., 2014). Therefore, the goal of this review is to critically discuss the molecular factors thought to contribute to the effects of androgens on skeletal muscle protein balance and to identify critical areas of future research required for the continual progression towards the development of androgen-independent therapies.

2. Androgens

Androgens represent a class of hormones predominantly responsible for the development of male secondary sex characteristics including increased muscle mass (White et al., 2013a,b; Guyton, 2006). While females also synthesize androgens, circulating concentrations are much lower (Guyton, 2006), likely contributing to their smaller muscle mass. In males, androgens are synthesized in the Leydig cells of the testes using cholesterol as a precursor (Guyton, 2006). The adrenal cortex also produces androgen hormones, though the contribution of this alternative source to overall levels in males is thought to be negligible (Guyton, 2006). In contrast, this non-gonadal source in females accounts for a much larger portion of total androgen production (Guyton, 2006). Testosterone and its reduced metabolite, 5α-dihydrotestosterone (DHT), are the two most prominent anabolic androgens and can be produced locally in skeletal muscle from precursor androgens (i.e. Dehydroepiandrosterone; DHEA) via the enzymes 3β-hydroxy-steriod dehydrogenase, 17β-hydroxy-steriod dehydrogenase, or 5α-reductase (Sato et al., 2008; Aizawa et al., 2007). However, the *in vitro* concentrations of DHEA and/or testosterone precursors needed to induce this conversion were in the micromolar range and well above normal physiological concentrations, indicating that further *in vivo* studies are required to confirm whether this occurs when circulating concentrations are much lower than those used *in vitro* (Hopper and Yen, 1975; Velders and Diel, 2013). In general, circulating total testosterone values between 17 and 35 nmol/l are considered to be in the normal physiological range for males (Velders and Diel, 2013; Sader et al., 2003). Despite circulating concentrations of androgens being most frequently reported, concentrations within the tissues may also be important. For instance, evidence suggests that concentrations of androgens in skeletal muscle, rather than in circulation, is more predictive of strength and muscle cross sectional area at least in older men (Sato et al., 2014). Further, while not conducted in muscle cells, intracellular androgen concentrations in cultured prostate cancer cells differ from those values observed in the surrounding culture media. When extrapolated to skeletal muscle, this suggests that measurement of hypogonadal or physiological concentrations of androgens in circulation may not be representative of those levels within skeletal muscle (Sedelaar and Isaacs, 2009; Wu et al., 2013).

The most recognized androgen mechanism of action is through binding to the cytosolic androgen receptor (AR) (Guyton, 2006). Upon androgen binding, the AR translocates to the nucleus where it interacts with the androgen response element (ARE) of target genes to alter gene transcription (both positively and negatively) (Guyton, 2006). However, the role of this mechanism *in vivo* has been questioned since the dissociation constant (K_d) of testosterone or DHT for the androgen receptor was estimated to be ~2–5 nM (Wilson and French, 1976), which can be lower than androgen concentrations found in hypogonadal males (i.e. <17 nmol/l) (Velders and Diel, 2013). Thus, the receptor could be saturated even in a hypogonadal state, suggesting that alternative androgen-mediated mechanisms exist. Indeed, testosterone administration to L6 myoblasts in culture altered signaling events within 20 min of exposure (Wu et al., 2010); a time frame which is likely to be shorter than the traditional AR-mediated changes in gene transcription; illustrating the presence of alternative mechanisms of action. However, these alternative mechanisms remain poorly defined and require further attention representing an avenue for pharmacological intervention.

3. Regulation of protein balance

Skeletal muscle mass is regulated in part by the coordinated balance between rates of muscle protein synthesis and muscle protein breakdown. In healthy individuals, where muscle mass is maintained, these two processes wax and wane throughout the diurnal cycle in response to anabolic (i.e. nutrient consumption) and catabolic (i.e. fasting) stimuli (Phillips et al., 1985). Conversely, a long-term shift in this balance favoring net protein synthesis results in muscle hypertrophy while a long-term shift favoring net protein breakdown results in muscle atrophy (Phillips et al., 1985). These concepts and the molecular regulation of each have been reviewed elsewhere and therefore are only briefly summarized (Gordon et al., 2013; Hornberger, 2011; Kimball and Jefferson, 2010; Laplante and Sabatini, 2009; Ma and Blenis, 2009; Milan et al., 2015a,b; Sandri, 2010, 2013; Goodman and Hornberger, 2014).

In general, the increase in protein synthesis following anabolic stimuli requires signaling through the mechanistic target of rapamycin in complex 1 (mTORC1) (Dickinson et al., 2011; Drummond et al., 2009). Signaling through mTORC1 regulates mRNA translation initiation as well as peptide chain elongation through phosphorylation of at least two known substrates termed the 70 kD ribosomal protein S6 kinase 1 (p70S6K1) and the eukaryotic initiation factor 4E (eIF4) binding protein 1 (4E-BP1) (Kimball and