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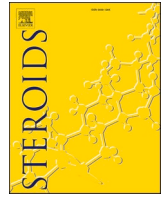
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An overview on androgen-mediated actions in skeletal muscle and adipose tissue

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ABSTRACT

Androgens are a class of steroid hormones primarily associated with male sexual development and physiology, but exert pleiotropic effects in either sex. They have a crucial role in various physiological processes, including the regulation of skeletal muscle and adipose tissue homeostasis. The effects of androgens are mainly mediated through the androgen receptor (AR), a ligand-activated nuclear receptor expressed in both tissues. In skeletal muscle, androgens via AR exert a multitude of effects, ranging from increased muscle mass and strength, to the regulation of muscle fiber type composition, contraction and metabolic functions. In adipose tissue, androgens influence several processes including proliferation, fat distribution, and metabolism but they display depot-specific and organism-specific effects which differ in certain context. This review further explores the potential mechanisms underlying androgen-AR signaling in skeletal muscle and adipose tissue. Understanding the roles of androgens and their receptor in skeletal muscle and adipose tissue is essential for elucidating their contributions to physiological processes, disease conditions, and potential therapeutic interventions.

1. Introduction

Androgens are mammalian endogenous cholesterol-derived steroid hormones, that include testosterone (T), dihydrotestosterone (DHT), androstenedione, dehydroepiandrosterone, and dehydroepiandrosterone sulfate [1]. They are synthesized and secreted under the control of the hypothalamo-pituitary system in various tissues, primarily by the testis in males, in relatively small quantities by the ovaries in females, along with the adrenal cortex, brain and skin in both biological sexes, and the placenta [2-5]. Although the actions of androgens and their secretion dosage are highly sex-related [6], they exert mutual pleiotropic effects in both female and male mammals, that are intrinsic for the maintenance of their reproductive competency, muscle size and strength, adiposity and metabolism, cardiovascular health, bone remodeling and mass retention, as well as brain function [7]. For centuries, the nature of these substances remained undefined, until they were discovered by the German scientist Adolf Butenandt in the early 1930s [8]. This ushered in the search of what is today known as testosterone, which together with its potent derivative, DHT, the predominant and most active androgen form, are the principal androgens in the circulation of mature male mammals [9]. Despite the presence and

necessary activity of testosterone regardless of the sex [10], its functions have been shown to be crucial for the male reproductive and cerebral health [11,12], with comparatively more drastic effects associated with the disruption of its secretion [12,13], and increased risk of mortality, regardless of the etiology of disease [14]. Moreover, in spite of similar androgen receptor (AR) levels in both females and males, AR absence selectively predisposes men to glucose and energy metabolism dysfunctions, excess adiposity, insulin resistance, and type-2 diabetes [15].

During almost the whole lifespan of a male individual, testosterone is secreted and regulates the homeostasis of not only reproductive, but also somatic tissues, including those of mesenchymal origin. This carefully orchestrated chronology of androgen actions is generally mediated by AR, a nuclear receptor encoded by a single gene located on the X chromosome, that belongs to the nuclear receptors' superfamily [16]. Upon activation by testosterone and/or its potent metabolite DHT, AR is subjected to binding-induced conformational changes, that dissociate it from chaperone proteins and expose its nuclear localization sequence. The AR-androgen complex then translocates to the nucleus, where it forms dimers that recognize a palindromic dihexameric DNA motif called the androgen response element (ARE), which controls gene expression through the recruitment of coactivators or corepressors [17]

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(Fig. 1). In addition to this DNA-binding-dependent mechanism, androgens are also known to regulate non-genomic processes, involving rapid, membrane-mediated non-transcriptional mechanisms in the cytoplasm [18] (Fig. 1). Although a lot on androgen signaling is being uncovered, the entire intricate picture of their actions and the mechanisms which they underly, especially in metabolism homeostasis maintenance, have not yet been fully explained.

In this review, we provide a comprehensive treatise of the role of androgen hormones, mainly through their receptor, in two key metabolic tissues, namely skeletal muscle and adipose tissue. After briefly introducing androgen biology fundamentals, we examine their pluripotent systemic and metabolic functions in either sex. Then, we depict skeletal muscle and adipose tissue androgen-dependent biology, by exploring methodologies and models that have been instrumental in improving and broadening our knowledge on the roles of testosterone and its receptor, in regulating both tissues differentiation and homeostasis. In summary, we lay out how progress in discovering and characterizing androgen signaling in skeletal muscle and adipose tissue, as well as in characterizing tissue core specific functions beyond their systemic effects, will have vast implications on our understanding of skeletal muscle and adipose tissues development, functions and integrity maintenance.

2. Effects of androgens on muscle physiopathology

2.1. Androgens and skeletal muscle

Beyond sexual purposes, androgens play critical roles in the regulation of body systems and metabolism in both females and males. Their mediated actions have been observed and described in various tissues, such as ovaries, endometrium, vulvovaginal tissue and breasts in females; and in numerous metabolic tissues including kidney, pancreas, liver, bone, adipose tissue and muscle in males. Skeletal muscle is a complex and highly specialized tissue that accounts for approximately the one-third of the adult mammalian body mass [19]. It constitutes a primary site for nutrients storage, energy use and locomotion. In both females and males, muscle homeostasis is maintained by cellular and endocrine interactions, including androgen signaling [20]. In men, testosterone is the main androgen in skeletal muscle [21], and its receptor is expressed in both satellite cells [22] and myofibers [15], the stem and specialized muscle cells, respectively, alongside other cell

types including the fibro-adipogenic progenitors and motoneurons [23], where its functions are becoming understood at basic levels, but a lot remains to be fully investigated.

2.1.1. Effects of androgens on skeletal muscle

Androgen main effects in skeletal muscle are anabolic and include growth, control of strength [24], contractile and metabolic functions [15], through various mechanisms. In males, during embryonic development, androgens and their receptor are mandatory for perineal muscles formation [25], and mediate the inguino-scrotal descent and the differentiation of the cremaster muscle [26]. However, they are unessential for limb muscles establishment, but crucial for their growth during post-natal life [27]. These androgen-dependent features are sex-related. For instance, experiments lead on AR-null female mice showed that they developed normal muscle with unaltered functions [27]. At puberty, testosterone peaks with a nearly 30-fold increase in its production [28]. This surge has dramatic effects on the musculoskeletal system, which are translated by rapid accumulation of muscle mass and acquisition of muscle strength [29,30]. These actions are androgen-mediated through different pathways. Muscle mass could be increased by promoting the differentiation of multipotent mesenchymal stem cells into myogenic lineage, stimulating muscle protein synthesis [31] and activating satellite cells [32]. Androgens can also stir whole-body protein synthesis and inhibit proteolysis, resulting in a net anabolic effect on the muscle, increasing its mass and maintaining it [33]. The age-related decline in serum testosterone levels is associated with reduced muscle mass and strength [34]. In attempts to reverse this effect, androgen supplementation has been clinically administrated for elderly men to promote muscle mass gain [35]. However, many studies in adult men have reported that muscle strength decline is not necessarily associated with serum testosterone levels decrease [24,36]. These androgen endogenous effects described here above can be mimicked by synthetic compounds that are used either in pathological, athletic or gender transition contexts to enhance skeletal muscle structure or functions [37-43].

In rodents, administration of supraphysiological doses of testosterone increased fiber cross section area and satellite cells number [44]. In adult rodents, muscle mass decrease was significantly prevented by testosterone treatment in muscles comprising fast-twitch fibers, such as tibialis anterior, extensor digitorum longus, and plantaris, when compared to others comprising slow-twitch fibers, such as soleus [45].

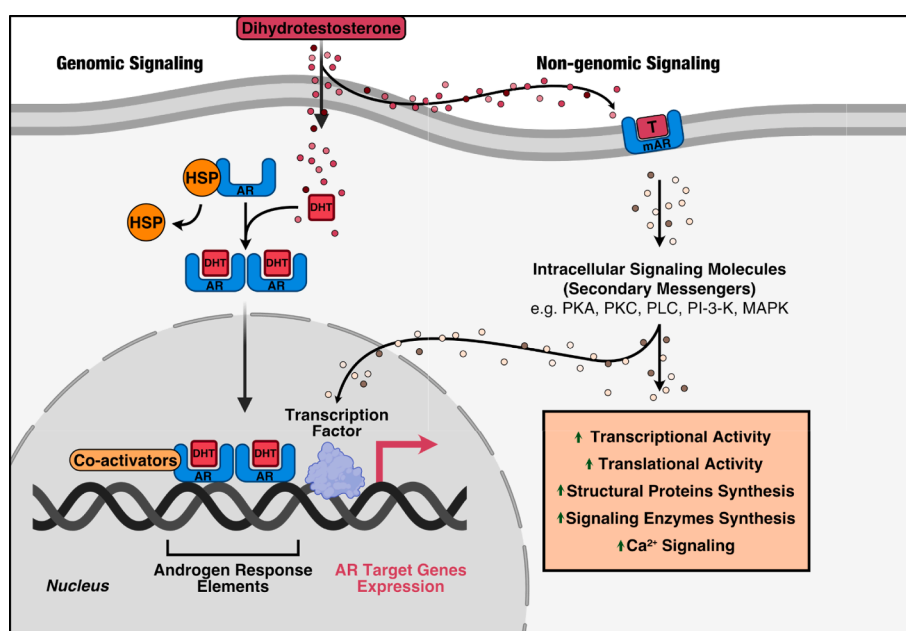


Fig. 1. Genomic and non-genomic androgen-mediated signaling pathways. Genomic signaling is mediated by ligand (e.g. dihydrotestosterone, DHT) entry in the cell through the cytoplasmic membrane. Upon ligand binding to the androgen receptor (AR), the AR-heat shock protein complex (HSP) retaining AR in the cytoplasm dissociates. AR bound to its ligand translocates to the nucleus as a homodimer, and exerts its transcriptional activity by binding androgen response elements (AREs), in association with coactivators and other transcription factors. Non-genomic signaling is characterized by ligand binding to a membranal AR (mAR). Consequently, secondary messengers induce a cascade of intracellular signaling such as protein kinase A (PKA), protein kinase C (PKC), phospholipase C (PLC), phosphoinositide 3-kinases (PI3Ks) or mitogen-activated protein kinases (MAPKs). These latter will promote processes such as transcriptional and translational activities, structural protein and signaling enzyme synthesis, and other signaling pathways such as calcium mediated signaling. The figure was created with BioRender.com (www.biorender.com).

Interestingly, in aging contexts, reversal of geriatric muscle phenotype, including decreased muscle mass, and impairment of muscle ultra-structure, could be attenuated by administrating testosterone, and the systemic environment that sustains muscle growth was restored [46]. In limb muscles, androgens activate numerous pathways that either stimulate protein synthesis and muscle hypertrophy, or inhibit catabolic pathways [47]. For instance, low testosterone levels were associated with a decrease in the muscle insulin like growth factor 1 (*Igf1*), a gene implicated in muscle mass regulation, and the protein kinase B (PKB) phosphorylation, the rate limiting enzyme of myofibrillar protein synthesis and many of its targets such as glycogen synthase kinase-3 beta (*Gsk3β*), proline-rich Akt substrate of 40 k_D (*Pras40*) and forkhead box O3 (*Foxo3a*) [48]. Noteworthy, FOXO3A transcriptional targets such as muscle RING-finger protein-1 (*Murf1*), atrogin 1, and regulated in development and DNA damage responses 1 (*Redd1*) were also affected [48]. In conclusion, skeletal muscle is a highly androgen-responsive tissue, and many aspects of its homeostasis are modulated by their actions.

2.1.2. Effects of androgens on satellite cells

Satellite cells are intrinsic resident skeletal muscle adult progenitor cells, the main function of which is to repair damaged muscle fibers, or replace them following fiber death during postnatal life [49]. Satellite cell hemostasis is controlled by interlacing environmental-triggered intercellular and hormonal signaling, that either maintains their quiescence for regenerative needs [50], or promotes their activation to heal the damaged muscle [51]. Among them, androgen signaling has been described as a main modulator of satellite cell physiological functions

and at different age stages [25], including quiescence at adolescence [52], and proliferation and differentiation at adulthood [53], consistent with the expression of AR in these cells [54].

Androgens are known to modulate satellite cells quiescence and proliferation in mammals [32]. By way of illustration, the peak of androgenic hormones at puberty in men is responsible for establishing a pool of satellite cells by promoting NOTCH signaling. This, consequently drives stem cells from proliferative to quiescent, or vice-versa, depending on environmental needs, but most importantly, by inhibiting in either way their commitment to the myogenic lineage [55]. This evolutionarily conserved pathway is highly coordinated in skeletal muscle, and its core anti-myogenic activity has been recently decoded. For instance, during early juvenile life, quiescent satellite cells, deprived from androgenic stimulus, exhibit a significant increased expression of *Notch* and of the receptor NOTCH intracellular domain (NICD). Elevated NOTCH signaling maintains the expression of paired box 7 (PAX7), a transcription factor that plays a crucial role in the formation and differentiation of skeletal muscle precursor cells during embryonic development, and preserves their stemness by inhibiting the expression of myoblast determination protein 1 (*Myod1*), and myogenin (*Myog*), two transcription factors that mark entry and differentiation into myogenic lineage [52,56,57]. The androgen-dependent NOTCH-induced quiescence in satellite cells is thus maintained by direct cell-to-cell contact between them and mature fibers, highly expressing delta-like 1 and 4 (DLL1, DLL4), the NICD ligands [52] (Fig. 2A). Testosterone peak at puberty induces the expression of mind bomb 1 (*Mib1*), a ubiquitin ligase that facilitates the ubiquitylation in satellite cells, and subsequent trans-endocytosis of the NOTCH ligands Delta-like 1 (DLL1) and/or Jagged 1 (JAG1) in myofibers, which

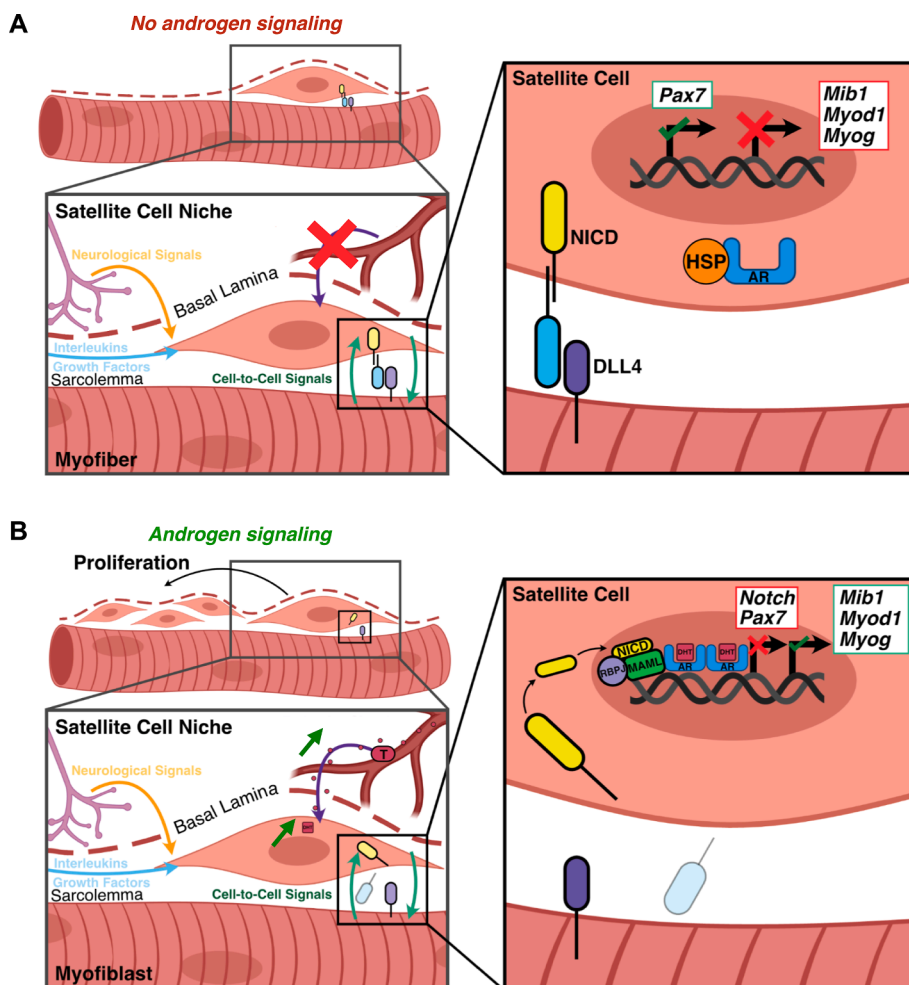


Fig. 2. Regulation of satellite cell homeostasis by androgen signaling. A. **Androgen-mediated stemness regulation.** Satellite cell stemness is maintained by various factors such as molecular signaling and mechanical stimuli. In the absence of androgen signaling, they highly express NOTCH, and are in direct cell-to-cell contact through intracellular domain of Notch (NICD) with myofibers expressing ligands Delta-like 4 (DLL4). This maintains the expression of paired box 7 (*Pax7*) and inhibits that of mind bomb 1 (*Mib1*), and consequently myogenic differentiation factors such as myogenic determination protein 1 (*Myod1*) and myogenin (*Myog*), preserving satellite cells in a quiescent state. B. **Androgen-mediated proliferation and differentiation.** In response to androgen signaling, ligand-receptor interactions between satellite cells and myoblasts trigger NICD intramembrane proteolysis, and release of the NICD into the cytoplasm. The latter translocates into the nucleus where it forms a complex with Recombination Signal Binding Protein for Immunoglobulin Kappa J Region (RBPJ), and members of the coactivator Mastermind-like (MAML) family. This represses the expression of paired box 7 (*Pax7*), and activates the transcription of mind bomb 1 (*Mib1*), which subsequently inhibits their NOTCH signaling and onsets the expression of myogenic differentiation factors such as myogenic determination protein 1 (*Myod1*) and myogenin (*Myog*). The figure was created with BioRender.com (www.biorender.com).

subsequently inhibits their NOTCH signaling and onset their differentiation [52]. This same case applies to regenerative demands [57,58] (Fig. 2B). In regenerative contexts, androgens indirectly interfere to recover the NOTCH pathway in satellite cells, by inducing the expression of *Mib1* in regenerating myofibers, that through direct cell-to-cell contact will inhibit their further differentiation and replenish their pool [52]. This will restrict in a number of stem cells the inhibition of NOTCH activity, which will maintain their undifferentiated state by preventing the expression of differentiation factors, and promote their self-renewal [52,57]. In addition to controlling quiescence and proliferation in satellite cells, androgen-mediated NOTCH signaling modulates a wide range of physiological aspects in satellite cells. For example, it safeguards them from spontaneous fusion with other cells by repressing myomaker (*Mymk*) expression, a myogenic fusion factor [57,59], and stimulates collagen V chain encoding genes that directly contribute to the build-up of their quiescent niche, and the micro-RNA mir-708 that anchors satellite cells to their anatomical location by targeting molecules involved in cell migration [52]. Quiescence exit and entry into myogenic lineage was also observed in castration in the absence of androgenic stimuli [60]. These observations indicate that, the hypothalamic–pituitary–testicular–androgens–MIB1–NOTCH pathway is a crucial regulatory mechanism for establishment of quiescent satellite cells at puberty, and during muscle regeneration at adulthood.

In adults, satellite cells' number linearly correlates with the change in testosterone serum concentration. For example, testosterone administration increases the number of satellite cells without elevating myonuclear count in myofibers in perineal muscles [61]. In the cases of androgens misuse or abuse, athletes who are under excessive androgenic supplementation exhibit a greater number of satellite cells and central myofibers nuclei, when compared to athletes who do not use testosterone-like substances, indicating activation and proliferation of satellite cells to form new fibers [62]. Moreover, female and male rodents pre-pubertally treated with testosterone, show an increased number of satellite cells and myofibers nuclei from newly fused differentiated cells in levator ani muscles [63]. In addition, testosterone treatment enhances satellite cells proliferation and differentiation in young and old mice during limb muscles regeneration [40]. This proliferation is indeed androgen-mediated since it was demonstrated that testosterone can activate quiescent satellite cells and promote their re-entry into the cell cycle [53]. This is consistent with findings in porcine muscles, for which castration reduced satellite cells proliferation, whereas administration of testosterone increased their number [64].

Proliferation of satellite cells could eventually lead to their subsequent differentiation and fusion to form new myofibers. Therefore, a negative control loop that restrains androgens' own anabolic effects through satellite cells, thus muscle hypertrophy in muscles, is established by androgens themselves. Any excess in androgen secretion in satellite cells will lead to a systemic response that will restrain their actions in these cells [65]. In addition, using a *Myod1*-CRE deleter strain, AR was shown to control strength and fiber type distribution in limb muscles, whereas it only affected perineal muscles' weight [65]. On the molecular levels, proliferation, differentiation and eventually post-maturation capacity to force generation, are mediated by a highly androgen-responsive gene, myostatin (*Mstn*), a strong inhibitor of skeletal muscle growth. In physiological conditions, androgens via AR promote *Mstn* transcription. MSTN signaling subsequently represses myogenic factor 5 (*Myf5*), and *Myog* [65] that play an important role in muscle development and regeneration [66]. Nevertheless, since the *Myod1* promoter is mainly active in myoblasts [67] and myofibers [68], the role of AR in satellite cells requires further investigation.

All together these results demonstrate that androgens, via their receptor, contribute to satellite cells stemness, activation, proliferation and entry into the myogenic lineage. They control through NOTCH signaling skeletal muscle stem cells' quiescence and/or activation, and through MSTN pathway their capacity to integrate the myogenic

lineage. However, it is still unclear through which mechanisms androgens in satellite cells contribute to muscle growth and regeneration, which requires the establishment of an exhaustive AR specific satellite cell knockout model that is exclusive to them.

2.1.3. Effects of androgens on myofibers

Myofibers are highly specialized skeletal muscle cells with specific contractile and metabolic capacities [69]. Contractility of myofibers is conferred by myofibrils in the muscle fiber cytoplasm, organized in sarcomeres, the smallest contractile unit responsible for force generation [70], which sustains countless vital functions for the mammalian physiology maintenance, such as breathing, locomotion, and mechanical digestion [71]. In addition to contraction, muscle fibers comprise metabolic functions including glycolysis, fatty acids beta-oxidation, and alpha-amino acids catabolism that are responsible for energy homeostasis and expenditure. Interestingly, both contractile and metabolic aspects of myofibers are controlled by androgens, through AR [15,72]. Although it has been demonstrated that AR culminates in male mice skeletal muscle between 11 and 15 weeks of age [15], the key cellular and molecular mechanisms that are androgen-regulated remain elusive.

To shed more light on the functions of AR, on global physiology in general, and in skeletal muscle in particular, many AR knock-out mouse models were established. For example, the androgen receptor knockout (ARKO) male mice, or AR-null mice [73], exhibited a female-like physiognomy, the weight of their muscles were reduced, as well as their fiber cross-sectional area and contractile strength [74]. In castrated mice, androgen depletion impaired muscle protein synthesis [75], and steered an imbalanced distribution in the fiber types [76]. All these evidences suggest that androgens, through their receptor, orchestrate skeletal muscle homeostasis.

In an attempt to increase our understanding of androgen specific actions in skeletal muscle, models with selective ablation of AR in mature fibers have been produced. Until today, only two models have been generated by the means of the cyclization recombinase-locus of crossing over of Phage 1 (CRE-*loxP*) system [77]. In 2009, the post-mitotic myocyte-specific AR knockout (mARKO) model [78] was established by crossing female mice, in which the exon 2 of the *Ar* gene is floxed by two *loxP* sequences [79], with male mice expressing the CRE under the control of the muscle creatine kinase promoter (*Mck*-CRE). In transgenic mice, CRE is selectively expressed in myofibers, but not in satellite cells or myoblasts from embryonic day 13 and on, and excises the floxed exon 2 in order to invalidate AR [78]. Phenotypically, mARKO male mice display significant weight decrease, conversion of fast fibers toward slow fibers, with no noticeable effects on strength or fatigue, suggesting that myocyte AR orchestrates muscle anatomical aspects [78]. In 2010, our laboratory generated the AR^{skm-/y} model [24], in which AR^{L2/L2} female mice, harboring *loxP* sites that flank exon 1 of *Ar*, encoding the AR N-terminal domain [80], were crossed with male mice that express CRE under the control of α -skeletal actin promoter (*Hsa*-CRE). In male transgenic mice, CRE is selectively expressed in myofibers, but not in satellite cells or myoblasts, from embryonic day 16 and on, and excises the floxed exon 1 in order to invalidate AR [24]. Our model presented a phenotype that was in contrast with the preceding mouse model [78], as we demonstrated that myofiber AR exerts two distinct muscle type-specific roles, in perineal and limb muscles. Case in point, our study showed that perineal-myofiber-AR transduces fiber hypertrophy during postnatal development, and maintains fiber size at adulthood, in bulbocavernosus (BC) and levator ani (LA) muscles [24]. We hypothesized in this context that local androgen-induced insulin-like growth factor IEa (IGF-IEa), stimulates muscle myofibrillar protein synthesis through activation of AKT/mTORC1 signaling. Another non-genomic pathway was also described, in which androgens bind to surface receptors, leading to AKT/mTORC1 activation [81]. In limb muscles, we demonstrated that myofiber-AR is neither essential for muscle growth during postnatal life, nor for muscle mass maintenance during adulthood [24]. However, ablation of limb-myofiber-AR had a severe

impact on overall architecture and structural units of skeletal muscle. Not only muscle fiber size and mass were reduced, but also the strength of fast- and intermediary-twitch fibers, since myofibrillar organization was altered in sarcomeres [24]. Overall, we showed that androgens play distinct muscle type-specific roles, and are critically involved in maximal force production, and control intrinsic contractile functions of fast- and intermediary-twitch hindlimb skeletal muscles [24].

Beyond the scope of our own investigation, a separate study conducted on female mice divulged supplementary implications of androgens in skeletal muscle [72]. Interestingly, through their receptor, androgens directly control muscle strength by specifically targeting a newly identified splicing variant of myosin light-chain kinase 4 (*Mylk4*) in myofibers. Surprisingly, androgens can also elicit the activation of AR in extra-myofiber cells or tissues to regulate muscle mass, proving that androgen-mediated actions in skeletal muscle can be muscle-context-independent [72].

These findings deliver compelling evidence of androgens anabolic effects in skeletal muscle, but the molecular events underlying these actions remained largely unexplored. Hence, we furtherly investigated the role of limb-myofiber-AR and demonstrated that in male myofibers, AR exerts through direct targets, contractile and metabolic functions [15]. For instance, AR invalidation induced sever abnormalities in muscle structure, characterized by disorganization of the cytoskeleton elements, including enlarged sarcoplasm and disrupted sarcoplasmic reticulum [15]. Remarkably, modulators of muscle contraction were also drastically impaired. The latter include disordered triad, sarcomeres with loss of myofibrils, decreased amounts of myofilaments and ruptured of Z-lines [15]. On the molecular levels, we showed that AR directly controls the expression of numerous genes involved in the contractile cytoskeleton, and in muscle non-contractile cytoskeleton such as ryanodine receptor 1 (*Ryr1*), dystrophin (*Dmd*), desmin (*Des*), and caveolin 3 (*Cav3*). Genes that were also directly controlled by AR included those that modulate the extracellular matrix such as *Itgb4*, *Itgb5*

and *Itgb7*; *Mylk4* and *Myom1* that contribute to muscle strength; and *Cacnai* and *Cacng7* encoding subunits of calcium voltage-gated channels [15] (Fig. 3A). Thus, these changes correlate with decrease of muscle force, and diminished contractile potency reported upon AR loss [15,24,82]. Overall, we demonstrated that androgens, through their receptor in myofibers, control pathways required for contraction and maximum force production in skeletal muscles via a direct control of target gene expression.

In addition to muscle structural controls, we unveiled that limb-myofiber-AR controls glucose, fatty acids (FA), and amino acids (AA) usage. First, we showed that androgens maintain glycolytic metabolism in limb muscles, and shield male individuals from type-2 diabetes [15]. Upon AR invalidation in myofibers, we observed that blood glucose and insulin levels were higher, and mice became glucose intolerant and insulin resistant [15]. On the molecular levels, we unraveled various AR direct targets including the rate-limiting enzyme of glycolysis, [83] hexokinase 2 (HK2). Consequently, hexokinase activity decreased in the absence of AR [15] (Fig. 3B). Noteworthy, female individuals did not exhibit any noticeable alterations [15]. Altogether, our data show that androgens, through their receptor in myofibers, control glucose uptake and glycolytic capacities, and prevent the development of type-2 diabetes in male mice.

Second, our study revealed that androgens via AR limit free fatty acid uptake in myofibers by decreasing the expression of the fatty acid transporter *Cd36*, as well as that of genes encoding enzymes of beta-oxidation [15], a significant source of metabolic energy from oxidation of fatty acids that occurs in mitochondria and provides ATP for the myocytes [84], such as *Acox1*, *Acads*, *Acadl*, *Echs1* and *Hadhb* [15]. We also unraveled that AR expression is required for FA-CoA import to the mitochondria to prevent their accumulation in cytoplasm, by increasing the expression and activity of the triglyceride hydrolase lipoprotein lipase (LPL) and the transcript levels *Pnpla2*, encoding the lipase adipose triglyceride lipase (ATGL) that catalyzes the conversion of triacylglycerides to diacylglycerides and FA-

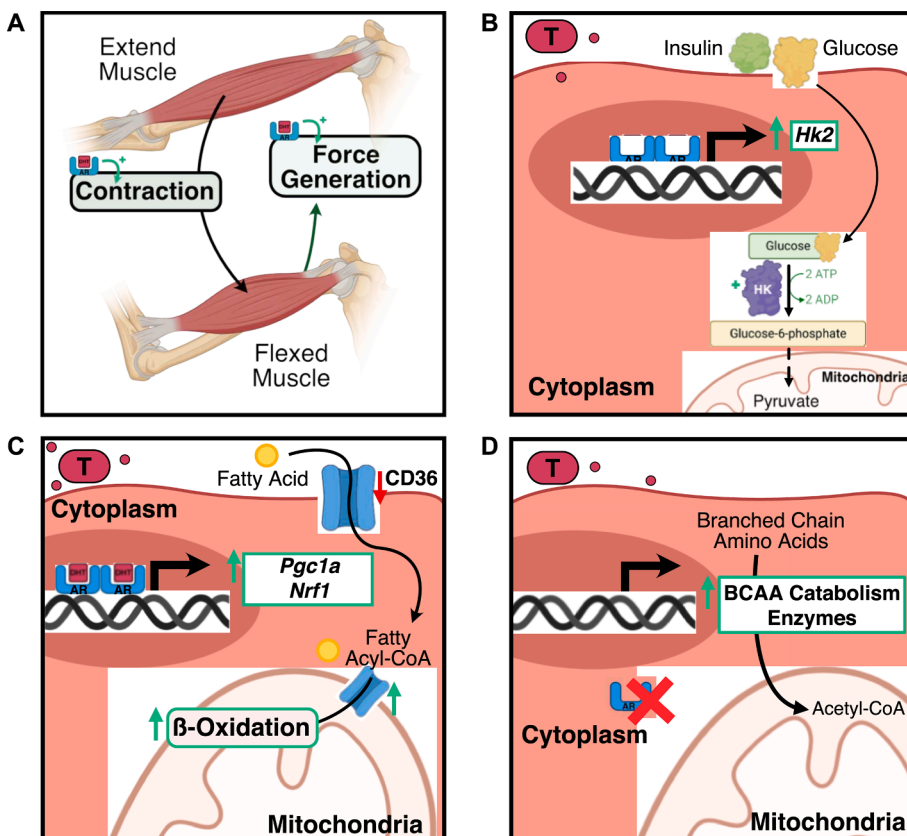


Fig. 3. Regulation of myofiber functions by androgen signaling. Androgen signaling in myofibers mainly control four main aspects. (A) First, it modulates contraction and force generation by inducing the expression of calcium (Ca^{2+}) channels and of various components of the contractile and non-contractile cytoskeleton. (B) In addition, they prevent the emergence of type-2 diabetes by increasing the expression of hexokinase (*Hk2*), thus facilitating glucose entry, catabolism and storage within the myofiber. (C) AR limits fatty acid import to the myofiber by the modulation of *CD36* expression. It also promotes the expression of *Pgc1a* and *Nrf1*, as well as the import of fatty acids to the mitochondria, thereby activating their oxidation. (D) Finally, the absence of AR in myofibers promotes the branched chain amino acids (BCAA) catabolism to generate acetyl-CoA. The figure was created with BioRender.com (www.biorender.com).

CoA, a beta-oxidation product [15] (Fig. 3C). Interestingly, we identified *Pgc1a* and *Nrf1*, two major coordinators of muscle oxidative metabolism and mitochondrial activity [85], as direct AR targets. We hypothesize that their reduced expression could diminish mitochondrial respiration in AR-deficient fibers.

Finally, we established that the loss of androgens signaling in myofibers promote amino acid catabolism and oxidative stress [15]. Our data revealed that absence of myofiber-AR decreased levels of lysine, leucine, isoleucine and valine, explained by the induction of transcript levels of the rate-limiting enzymes of branched-chain amino acids (BCAA) catabolism *Bcat2* [15] (Fig. 3D). We also showed that glutamate homeostasis was impaired in the absence of AR, leading to an increase in NH_3 content, and affecting the activity of mitochondrial terminal complexes by promoting oxidative stress. Our expanded metabolomics investigations also uncovered that ROS production was limited by androgens in myofibers [15].

These findings are in concordance with clinical observations made in patients with androgen inefficiency caused by disease and/or aging, where structural or functional muscular impairments could be partially or entirely rescued by androgen administration [86-88], improving tissue density and protein synthesis capacity, and subsequently mechanical and metabolic wellness in individuals [89]. In agreement, previous studies showed that AR-null male mice were characterized by increased muscle transcript levels of *Slc2a4*, *Hk1*, *Pfk* and *Pkm*, as well as decreased energy expenditure and lipolysis with late-onset obesity [90]. Moreover, a progressive reduction in insulin sensitivity and in glucose tolerance was observed in those mice, together with an acceleration of weight gain, hyperinsulinemia, and hyperglycemia, and increased triglyceride content in skeletal muscle and liver [91]. Epidemiological studies have shown that low testosterone levels are associated with obesity, impaired glucose tolerance, insulin resistance, an adverse lipid profile, decreased $\text{V}_{\text{O}_2\text{max}}$ and oxidative phosphorylation gene expression [92]. Conversely men with metabolic syndrome and type-2 diabetes have a high prevalence of hypogonadism [93]. Altogether, these data indicate that the development of type-2 diabetes in these conditions might be preferentially caused by impaired androgen signaling in myofibers, whereas dyslipidemia and obesity might result from its disruption in adipose tissue and/or liver.

Interestingly, in the context of Kennedy's disease (K_D), also known as spinal and bulbar muscular atrophy (SBMA), an X-linked recessive neuromuscular disease induced by glutamine expansions (CAG) in the AR N-terminal region [94], it was believed that the poly-Q-AR exerted cell toxicity [95], likely causing death and neuronal dysfunction [96,97]. Patients with SBMA suffer from metabolic alterations (e.g. glucose intolerance and hyperlipidemia) [98], reduced muscle force, associated with a switch in fiber-type composition, disrupted muscle striation, altered calcium dynamics in response to muscle contraction, and aberrant expression of excitation-contraction coupling (ECC) machinery genes [99], along with impaired myotube fusion, and altered myofiber contractile structures [100-102]. Corresponding poly-Q expansions in mice impaired glycolysis with decreased *Hk2* expression, and altered mitochondrial functions in their muscles [103], which resembles the phenotype of $\text{AR}^{\text{skm-}/y}$ mice, providing compelling evidence that the underlying cause of SBMA lies in disrupted transcriptional mechanisms rather than the toxicity associated with the poly-Q AR protein. Further investigation is warranted to explore the impact of the N-terminal extension on AF-1 function and the subsequent recruitment of co-regulators. Thus, clinics is shifting in diagnosis towards altered muscle, and not neuronal biomarkers, since the main novel described phenotype is associated with incapacity of muscle fibers differentiation and altered transcriptional profiles [102], with high creatine kinase concentrations, an enzyme associated with muscle damage [104], and low creatinine levels, necessary for protein synthesis and muscle mass maintenance [102,105].

In summary, androgens control through various mechanisms, via their receptor in myofibers, muscle mechanical and energetic functions,

as well as whole-body glucose metabolism.

3. Effects of androgens on adipose tissues physiopathology

Adipose tissue is an important regulator of energy balance and nutritional homeostasis and has recently been recognized as a complex endocrine organ [106]. It characteristically consists of adipocytes along with other cell types such as preadipocytes, macrophages, neutrophils, lymphocytes, endothelial cells, mesenchymal stem cells, and pericytes [107,108], which constitute the stromal vascular fraction (SVF) of adipose tissues [108]. On the basis of morphology and function, there are three types of adipocytes: brown, white, and beige. White adipose tissue (WAT) is mainly composed of white adipocytes that store energy in the form of triglycerides. In contrast, brown adipocytes that reside in discrete brown adipose tissue (BAT) depots, such as the interscapular area in rodents, and beige adipocytes that are intermingled with white adipocytes in WAT, metabolize triglycerides to dissipate energy in the form of heat via the thermogenic factor uncoupling protein-1 (UCP1) [109]. Structurally, white adipocytes have a unilocular lipid droplet, while brown and beige adipocytes have multilocular droplets, along with a high mitochondria number. As an endocrine organ, adipose tissue secretes several hormones, cytokines, and metabolites, collectively known as adipokines, that are known to regulate a variety of physiological processes [109,110]. Adipocyte differentiation and metabolism are regulated by several transcription factors, the key ones being PPAR γ , C/EBP α , EBF1, and MYC [111]. Of the vast array of factors involved in the pathophysiology of adipose tissue, this review focuses specifically on the involvement of androgens and androgen receptor (AR) in the context of signaling, proliferation/differentiation, and metabolism in adipose tissues, along with excerpts on topics such as Polycystic ovary syndrome (PCOS) in females. The model organisms discussed are largely males, unless stated otherwise, due to the large disparity in male and female studies discussed in the literature.

3.1. Androgen circulation, expression and signaling of AR

Human adipose tissues contain androgens in much higher concentrations than peripheral blood [112]. The concentration of DHEA and androstenedione in adipose tissue is higher than that of testosterone and DHT, despite the fact that the latter are known to be the most potent forms of androgen [112]. The androgen receptor (AR) is known to be expressed in adipocytes as well as in other cell types such as preadipocytes [113] and macrophages [114] within adipose tissues. Adipose tissues can be visceral (VAT; lining internal organs) or subcutaneous (SAT; beneath the skin), depending on their localization within the body [115]. AR levels are higher in VAT than in SAT [116], which is also the case with AR expression in human preadipocytes from both origins [117]. Androgens and their derivatives appear to have different interactions with AR, with testosterone and DHT showing the strongest androgen activity. The activity of certain androgens, such as androstenedione, can be altered depending on the presence or absence of other androgens in the vicinity, thus functioning either as an agonist or antagonist for AR [118]. Besides, the sensitivity and transactivation of AR both *in vitro* and in humans have been associated with the length of polymorphism of [CAG] trinucleotide repeats in the AR gene [119-121], thus portraying its role in adipose tissues along with parallel studies discussed in skeletal muscle. AR works in concordance with a wide variety of cofactors [122], which together influence AR-regulated gene expression and can serve as a mechanistic insight into the complex array of transcriptional responses generated by AR. For instance, ARA170, a type II coregulator known for exerting its role through AR conformational changes and stabilization, serves as a coregulator for both AR and PPAR γ [123]. Thus, cross-interactions between AR, ARA70, and PPAR γ could potentially influence the physiology of adipocytes. However, little is understood about these coregulators in relation to adipose tissues. According to the pertinent literature on androgen action in adipose

tissues, it appears that the adipose tissue is a key site for androgen uptake, metabolism, and action [124] and that the androgen availability in adipose tissues could be modulated by the local synthesis and inactivation of potent androgens by a number of steroidogenic and steroid-inactivating enzymes identified from human studies [124]. Similarly, AR in adipose tissues seems to have sex-specific and depot-specific roles cited in literature elsewhere [125] as well as in this review. Numerous physiological processes that AR is found to be involved in will be covered in the following sections. While androgens have been known to have both genomic and non-genomic roles [126,127], the sections pertaining to androgen roles in adipose tissue shall be restricted to the genomic functions due to the lack of sufficient and relevant literature in the non-genomic context.

3.2. Role of androgen in proliferation and differentiation of preadipocytes and mesenchymal stem cells

To obtain mature and functional adipocytes, mesenchymal precursor cells first commit to becoming preadipocytes, which then stop their growth to undergo terminal differentiation and produce adipocytes [128]. Studies performed in human and rat preadipocytes show that, while AR mRNA expression is strongly upregulated during the induction of differentiation, AR protein expression decreases in adipogenesis [113], suggesting complex post-transcriptional regulation of AR.

In the case of castrated rats, androgens have depot-specific effects on proliferation and differentiation. Castration increased both proliferation capacity and differentiation of epididymal preadipocytes in culture, but in the case of perirenal preadipocytes, while castration increased proliferation, the differentiation capacity was reduced [129]. In the same studies, testosterone supplementation reversed the proliferation changes in epididymal preadipocytes but not in perirenal. Interestingly, proliferation and differentiation were completely unaffected in subcutaneous preadipocytes, regardless of castration or testosterone supplementation [129]. However, the literature is conflicted in this aspect, since another study suggests that the treatment of either testosterone, DHT, or 17 β -estradiol failed to affect the growth rate of preadipocytes in culture from male rats [130]. This could be due to several potential reasons, such as different culture conditions, age of mice, duration, and dosage of treatment, and so further analysis is needed in this aspect.

DHT and testosterone seem to have inhibitory effects on primary preadipocytes from the subcutaneous and visceral compartments in both men and women [131]. DHT inhibits adipogenic differentiation in human mesenchymal stem cells (hMSCs) and preadipocytes from specific fat depots (subcutaneous, mesenteric, and omental) in culture through an AR-mediated pathway, but it does not affect their proliferation [132]. In hMSCs, testosterone and DHT effectively counteracted the adipogenic effect of rosiglitazone, a thiazolidinedione belonging to a class of anti-diabetic drugs [133] that has been shown to promote adipogenesis [134]. Similarly, androgen exposure of human adipose stem cells (hASCs) isolated from subcutaneous abdominal adipose tissue in women impaired commitment to preadipocyte formation and/or subsequent differentiation into adipocytes [135]. The responses to androgens observed for mouse preadipocyte cell lines 3T3-L1 and C3H10T1/2 are similar. Indeed, testosterone, DHT, and DHEA prevented *in vitro* differentiation of 3T3-L1 and C3H10T1/2 cell lines into the adipogenic lineage through an AR-dependent pathway. This prevention is characterized by reduced expression of adipogenic genes, including *PPAR γ* , *C/EBP α* , and *aP2*, along with promoting their commitment to the myogenic lineage [136] or the osteogenic lineage [137]. The reason for these multivariant lineages from adipose precursor cells could be due to several reasons, including differences in the differentiation medium and other culture conditions. Similar to the cell lines discussed above, testosterone and DHT also inhibit differentiation in primary brown adipocytes and brown adipose cell lines in rodents [138]. The potential mechanism by which androgens inhibit adipogenesis has been cited in several studies. Androgen treatment could induce the expression of

IGF1, a myogenic and anti-adipogenic stem cell factor, which in turn could increase the nuclear translocation of β -catenin and activate downstream Wnt signaling [139,140]. A recent study suggests that testosterone might not directly affect the adipose differentiation of 3T3-L1 cells. Instead, high concentrations of testosterone have been shown to regulate macrophage polarization in an AR-independent pathway, which then affects adipocyte differentiation [141]. In conclusion, androgens appear to be relevant for the proliferation and differentiation of adipogenic precursors in adipose tissue, as observed in multiple model systems (Fig. 4A).

3.3. Androgens and AR in metabolism and obesity

3.3.1. Androgen deprivation/supplementation models

Androgens are important determinants of body composition in men, promoting the growth of lean mass and suppressing the deposition of fat [142]. There have been several epidemiological studies that have observed a bidirectional relationship between low testosterone levels and obesity in men [143]. The presence of obesity can lead to reduced testosterone levels in men [144], and weight management by diet or surgery can increase testosterone levels proportionally to the amount of weight lost [145]. These alterations can originate either from issues of adipogenesis or fat storage. Male hypogonadism is a clinical syndrome consisting of symptoms and signs of androgen deficiency in combination with low serum testosterone [146]. A subcategory of male hypogonadism known as functional hypogonadism is usually due to hypothalamic-pituitary suppression by extragonadal diseases, with obesity being a very common cause [147]. There is an inverse relation between total serum testosterone and the amount of VAT (visceral adipose tissue) [148,149], which is also observed in age-related hypogonadism [89] and in cases of inherited testosterone deficiency [150]. Abdominal visceral obesity is in general more prominent in men with low baseline androgen levels, and testosterone supplementation in hypogonadal men decreases abdominal VAT accumulation and promotes metabolic improvements, including improved glucose homeostasis [151-153]. In the case of middle-aged men with abdominal obesity as well, testosterone treatment decreased VAT mass while making no difference in other fat depots [154]. Testosterone treatment in men with type-2 diabetes and hypogonadotropic hypogonadism decreases subcutaneous fat [155]. In the case of men with Klinefelter Syndrome, an aneuploid genetic condition with a 47,XXY karyotype and hypotestosteronemia, testosterone supplementation decreases abdominal fat [156]. In another study, testosterone treatment in middle-aged men with type-2 diabetes, visceral obesity, and mild androgen deficiency showed a reduction in body fat [157]. However, similar studies in women mostly appear inconsistent and controversial [124].

Studies using microarray and SAGE (serial analysis of gene expression) have demonstrated that 24 h of DHT treatment in the retroperitoneal adipose tissue of mice is sufficient to induce global changes in the transcriptional repertoire, affecting transcripts linked to processes like lipolysis, beta-oxidation, preadipocyte proliferation and differentiation, fatty acid synthesis, triglycerol synthesis, apoptosis, tissue morphology, and energy homeostasis [158]. Besides bulk observations of fat mass changes under the influence of androgens, studies were also reported on other physiological players such as glucose homeostasis and adipocytokines (signaling molecules released by adipocytes) (Fig. 4B-C). Testosterone in high concentrations was found to increase GLUT4-dependent glucose uptake in 3T3-L1 adipocytes by increasing the LKB1/AMPK signaling pathway [159]. Serum adiponectin levels are high in hypogonadal men and are reduced by testosterone therapy [160]. Testosterone treatment reduced plasma adiponectin concentrations in castrated mice, which also showed improvement in insulin sensitivity. In the same study, testosterone treatment in 3T3-L1 adipocytes reduced adiponectin secretion into the culture medium [161]. Metabolic syndrome is a set of conditions that increase the risk of health problems such as coronary heart disease and diabetes. There are many

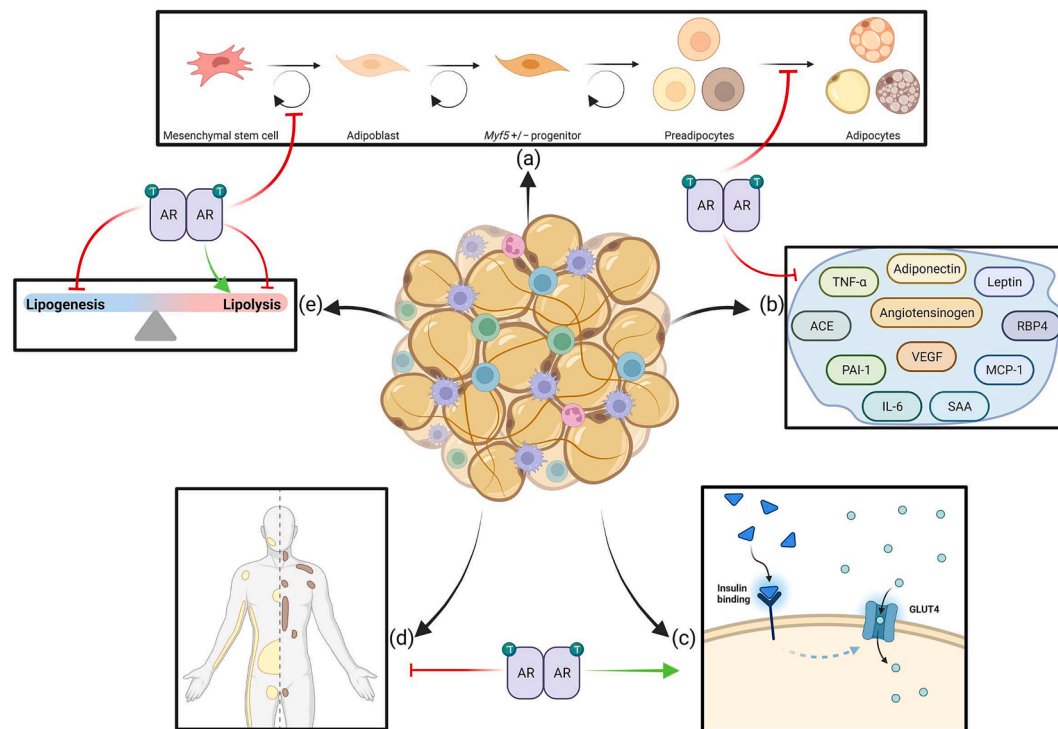


Fig. 4. Regulation of adipocyte physiology by androgen signaling. Androgens influence several aspects of physiology in adipose tissues, and while most of the observations discussed in literature are specific to the model organism, sex, and fat depot, a general consensus can still be drawn from them, keeping in mind that this necessitates further studies to be significant. Androgens (testosterone in this figure) interact through AR to mediate the following processes in adipocytes: (A) proliferation of mesenchymal stem cells and other adipose precursors along with adipogenesis. (B) the adipokine repertoire from adipocytes. (C) Insulin sensitivity, glucose uptake and glucose tolerance (D) body fat distribution (yellow depots: white adipose tissue; brown depots: brown/beige adipose tissue) (E) Lipogenesis and lipolysis. Adipose tissue is drawn in the center with other cells from SVF including macrophages, T cells and neutrophils. Red arrows indicate repressive effects and green arrows indicate beneficial/promoting effects. AR: androgen receptor; T: testosterone; GLUT4: glucose transporter type 4; Myf5: myogenic factor 5; TNF α : tumor necrosis factor alpha; RBP4: retinol binding protein 4; MCP-1: monocyte chemoattractant protein-1; SAA: serum amyloid A; IL-6: interleukin 6; PAI-1: plasminogen activator inhibitor-1; ACE: angiotensin converting enzyme; VEGF: vascular endothelial growth factor. The figure was created with BioRender.com (www.biorender.com).

studies that report that obesity, fat accumulation in the liver and intra-abdominal regions, and an increased risk of metabolic syndrome in men are associated with a decline in the levels of sex hormone-binding globulin (SHBG), which in turn leads to a decline in total testosterone levels [162-164].

Alterations in diet can also potentially affect the androgen action on adipose tissues, especially high-fat diet (HFD). For instance, treatment with testosterone reverted back to normal the metabolic status of VAT, such as the hypoxic state of the tissue, increased adipocyte size compared to a regular diet, reduced triglyceride accumulation, reduced glucose uptake, and adipocyte-specific genes in extracted preadipocytes, in a HFD-induced rabbit model of metabolic syndrome [165]. In another model of mice that underwent bone marrow transplantation from a donor with a knockout of AR in the bone marrow, HFD feeding increased fat mass and adiponectin levels, suggesting the involvement of AR in hematopoietic cells in influencing body fat distribution (Fig. 4D) [166]. Similarly, upon androgen deficiency via orchidectomy (ORX; removal of one or more testes) or genetic ablation [167], HFD promoted adiposity and serum leptin levels with respect to control mice, even though no such changes were observed in the regular diet. In addition, ORX-HFD mice exhibited increased sedentary behavior, increased hypertrophy, decreased mitochondrial content in white adipocytes, increased lipogenesis, decreased lipolysis, heightened glucose intolerance, increased IL-1 β serum levels, and impaired pancreatic secretion. Testosterone supplementation restored the castration effects on body composition and glucose homeostasis [167], which was confirmed by another study showing that testosterone treatment of ORX-HFD mice reduced weight gain, with decreases observed in visceral and subcutaneous fat mass as well as adipocyte size [168]. HFD in castrated mice also exhibited a

range of metabolic changes in epididymal white adipose tissues (epWAT) such as reduced WAT wet weight, reduced WAT/body weight ratio (Fig. 4D), induction of lipolysis, size reduction of adipocytes, reduced serum leptin levels, and increased stromal cell compartment in both diet groups, along with a few diet-specific changes [169].

Aside from diet, the key metabolic pathways that regulate the repertoire of various lipids and free fatty acids are lipogenesis, lipolysis, and thermogenesis (Fig. 4E). Testosterone supplementation in men decreased LPL activity and triglyceride uptake in subcutaneous and abdominal adipose tissue compartments [170,171]. Similarly, castrated rodents displayed reduced basal and catecholamine-induced lipolysis, both of which were completely normalized under testosterone supplementation. This was later confirmed via *in vitro* testosterone supplementation in human and rodent adipose tissues [172]. Testosterone in physiological concentrations can cause a depot-specific reduction of catecholamine-stimulated lipolysis in human SAT but not VAT [173]. Besides, depending on the concentration, DHT can either stimulate or inhibit the expression of HSL in human subcutaneous adipocytes [174]. The process of thermogenesis is usually discussed in the physiology of brown adipose tissue (BAT). In the case of Castrated mice and AR-knockout mice (global knockout under the CAG promoter), the β -adrenergic agonist CL316,243-induced elevation of body temperature was higher, along with an increased UCP-1 expression, a key mitochondrial gene involved in thermogenesis [175]. A similar study showed that castration in mice also increases basal lipolysis, triglyceride-derived fatty acid uptake, and decreases BAT lipid content [176]. However, these changes did not occur in the case of BAT-specific AR-KO. Besides, testosterone was found to reduce lipolytic activity [177] and decrease norepinephrine-induced UCP1 expression [178] in cultured brown

adipocytes of mice. In the case of humans, the density of BAT was found to be specifically correlated with the concentration of plasma androgens in men during the winter season [179]. In summary, androgens influence several aspects of metabolism, including adipocyte morphology, distribution of body fat across different depots, lipogenesis, lipolysis, thermogenesis, glucose uptake and tolerance, insulin uptake and tolerance, physical activity, and the adipokine repertoire.

3.3.2. Lessons from mouse models

AR-knockout (AR-KO) models, both global and tissue-specific, provide interesting insights into the metabolic relevance of androgen action in adipose tissues. One such global model, based on a DNA-binding impairment KO (AR-null mice), shows increased infrarenal and subcutaneous fat mass, reduced body mass and voluntary activity, decreased food intake, increased serum leptin and adiponectin levels, and altered lipid metabolism gene profile with respect to wild type mice [180], while two other such models have a slightly different phenotype where they exhibit late-onset obesity with increased body weight, decreased locomotor activity, hyperinsulinemia, leptin resistance, increased lipogenesis, reduced insulin-sensitivity, impaired glucose tolerance with age, reduced brown adipose tissue (BAT) thermogenesis, and increased levels of serum leptin and adiponectin [90,91,181]. A tamoxifen-inducible global AR-KO in mice showed that pre-pubertal but not post-pubertal AR inactivation resulted in an increase in fat mass [182], showing that AR might be very relevant to adipocyte metabolism before puberty in mice.

Unlike global AR-KO models, adipose tissue-specific AR-KO models provide a specified AR disruption, and the phenotype obtained from such KO can potentially be attributed exclusively to adipocyte metabolism. One such model was made by crossing mice expressing the CRE recombinase under the control of the adipocyte gene *aP2* with AR-floxed mice (floxed at exon 2). At 20 weeks of age, the mice were hyperleptinemic with respect to control, but they neither had leptin resistance as seen from exogenous leptin supplementation nor had any different body weight, adipose size, or adiposity index in terms of fat pad mass when compared to control [183]. It is important to note here, though, that the *aP2* promoter is also expressed in several other cell types in the brain, so the effects mediated by AR could be indirectly confounded by the brain via altering the specific effects of adipose tissue [184]. Meanwhile, another adipose tissue-specific AR-KO model was generated with CRE recombinase expressed under the *aP2* promoter, also with the AR floxed at exon 2 [185]. The mice, kept on HFD for 24 weeks, showed increased susceptibility to visceral obesity, hyperglycemia, and impaired insulin secretion. These discrepancies infer that the type of AR-KO can contextually influence the functionality of AR and hence provide a distinct set of metabolic alterations. Besides, a myocyte-specific AR-KO showed a decrease in intra-abdominal fat but no changes in the case of a satellite cell-specific AR-KO [65,78]. While these different AR-KO models provide valuable insight into the role androgens play in adipocyte metabolism, a deeper study on such mouse models needs to be performed to unravel AR's molecular mechanism of action in the various adipose tissues.

Aside from the aforementioned androgen deficiency models, a few interesting ones have also provided valuable information about androgen's effect on adipocyte metabolism. Androgen insensitivity syndrome, also known as testicular feminization, is a rare condition where the patients have a male karyotype (XY chromosomes) but are phenotypically female with a resistance to the action of androgen hormones [186,187]. In a testicular feminized model of mice, metabolic changes were observed that were depot-specific, such as reduced lipoprotein lipase (LPL) activity, increased activity for fatty acid synthesis enzymes, decreased PPAR γ expression, and decreased cholesterol efflux components in SAT, while in the case of VAT, they found decreased expression of steroyl-CoA desaturase-1 (which catalyzes oleic acid synthesis) and PPAR γ [188]. Finally, DHEA and testosterone treatment of the SVF fraction extracted from Otsuka Long-Evans Tokushima fatty rats, a

model for inherent obese type-2 diabetes, led to reduced body weight and epididymal fat weight as well as reduced cell proliferation. DHEA-induced effects were inhibited upon application of flutamide, an AR inhibitor, suggesting these effects are mediated via AR [189].

3.4. Androgens and AR in females – A perspective on PCOS and other metabolic alterations

Polycystic Ovary Syndrome (PCOS) is a complex endocrine disorder characterized by oligo-anovulation, hyperandrogenism, and polycystic ovaries on ultrasound. Interestingly, 70–80% of women with PCOS have high levels of circulating androgens, accompanied by comorbidities such as overweight or obesity [190]. The levels of androgens such as androstenedione and testosterone, as well as their ratios, appear to be relevant to the metabolic phenotype of women with PCOS [191], thus suggesting the involvement of steroidogenic enzymes in the pathophysiology of the disease. The levels of adipocytokines such as adiponectin, omentin-1, and leptin are reduced, with leptin levels being positively correlated with testosterone, body fat mass, BMI, waist-hip ratio, and metabolic abnormalities [192]. Hyperandrogenism due to PCOS leads to higher fat deposition in visceral WAT [193]. Non-obese PCOS women have an increase in catecholamine-induced lipolysis [194], and for both obese and non-obese women with PCOS and hyperandrogenism, adipocyte sizes appear to be larger than obese controls [195]. This hypertrophy of adipocytes may be due to imbalanced abilities of adipocyte storage or lipolytic activities [196], and it has been associated with high androgen levels within the subcutaneous adipose tissue [197]. High androgen levels are associated with an increased amount of fat localized on the trunk [198]. Thus, women with PCOS have more central obesity than weight-matched and BMI-matched controls [199].

Similar to the observations made in male studies, androgen action in female adipose tissues also alters their metabolic status. Testosterone treatment was observed to stimulate lipogenesis in women [200]. A combined exposure of mild hyperandrogenemia and a western-style diet in young adult female non-human primates induced hypertrophy of white adipocytes in omental VAT but not in white adipocytes of SAT, and this was associated with increased uptake and reduced mobilization of free fatty acids. This white adipocyte dysfunction could be attributed to multiple mechanisms, such as lipolysis suppression and reduced capillary density [201]. In contrast, prenatal testosterone treatment in female sheep led to a reduction in adipocyte size in VAT. Besides, their adipose tissue mesenchymal stem cells were found to have increased commitment to the adipocyte lineage and decreased differentiation to adipocytes at both pre-pubertal and post-pubertal time points [202]. In an AR-KO mouse model with an atherosclerosis-prone apolipoprotein E (apoE)-deficient background and provided with an HFD, female mice had increased body weight, body fat percentage, hepatic triglyceride levels, and reduced insulin sensitivity when compared to control mice with intact AR but also apoE-deficient and under HFD. This suggests that AR protects against diet-induced atherosclerosis in female mice [203]. In a study on non-human primates [204], *in vivo* androgen deprivation induced the appearance of very small, multilocular white adipocytes within WAT, which were restored to a unilocular phenotype with normal size upon testosterone replacement. In the same study, *ex vivo* culture of female adipocytes showed elevated basal fatty acid uptake upon treatment with androgens, which was even higher in the case of adipose tissues from ovariectomized females. Besides, testosterone supplementation in female rhesus macaques was found to reduce lipolysis and hormone-sensitive lipase (HSL) activity in WAT with respect to control in the luteal phase, and during menses, there was increased fatty-acid uptake and insulin signaling [205]. While not much has been discussed in the literature regarding the relation between androgen aromatization and body fat distribution, it has been observed that in women, visceral adiposity and adipose tissue dysfunction are associated with an increase in androgen catabolism or aromatization [206]. These studies together indicate that while androgens have a

significant role in the physiology of males, they also implicate several metabolic effects in females, including but not limited to adipocyte lipogenesis, lipolysis, and body fat distribution.

4. Conclusion

In conclusion, the roles of androgens through their receptor in skeletal muscle and adipose tissue have garnered significant attention in scientific research, and been subject to extensive investigations. The current findings highlight the multifaceted effects of androgens on both skeletal muscle and adipose tissue, emphasizing their significance in physiological processes related to body composition and metabolic homeostasis.

In skeletal muscle, androgens play a vital role in regulating muscle mass, fiber type composition, and overall muscular strength. Through satellite cells, androgens promote myogenesis. They facilitate muscle regeneration by enhancing satellite cell activation, proliferation, and differentiation, and promote muscle hypertrophy, by contributing to their expansion and the formation of new muscle fibers. In myofibers, they exert control over metabolism, structure and contraction of myofibers. They activate anabolic pathways, stimulate protein synthesis, enhance glycogen storage and promote the utilization of fatty acids as an energy source during exercise. In addition, androgens regulate calcium channels, thereby modulating muscle contraction and subsequently influencing its force. These effects collectively contribute to improved muscle performance and athletic prowess.

Similar to skeletal muscle, androgens also play a vital role in the pathophysiology of adipose tissue. They regulate both the proliferation of adipose stem cells and adipogenesis, altering the distribution of different adipocytes in the process. As for their metabolic role, they are involved in a plethora of processes ranging from the distribution of body fat, food intake, and physical activity/sedentariness, to cellular processes like thermogenesis, lipogenesis, lipolysis, glucose tolerance, insulin sensitivity, and the alteration of several adipokine levels. The culmination of studies discussed in this review implicates a contextual participation of androgens in adipocyte physiology, with observations altering across different model systems. Thus, while the general consensus suggests an overall beneficial role for androgens in adipocytes, it certainly warrants further studies to solidify this notion.

Understanding the roles of androgens in skeletal muscle and adipose tissue has significant implications beyond basic physiology. Dysregulation of androgen signaling is associated with various pathological conditions, such as muscle wasting disorders and metabolic disturbances. Thus, exploring the molecular mechanisms underlying androgen-mediated effects could provide valuable insights for the development of therapeutic interventions. Further research is needed to uncover the precise molecular pathways and downstream signaling events involved in androgen receptor activation in skeletal muscle and adipose tissue. Additionally, investigating the interplay between androgens and other hormonal systems, such as growth factors and cytokines, may yield a more comprehensive understanding of the complex regulatory networks at play. Unravelling androgen signaling in additional target tissues will open new avenues to develop AR ligands with increased selective activities for the treatment of various diseases.

Overall, the roles of androgens through their receptor in skeletal muscle and adipose tissue encompass a wide range of physiological processes, impacting muscle growth, metabolism, and adipose tissue homeostasis. Continued investigation in this field holds promise for advancing our understanding of these tissues and potentially uncovering novel therapeutic strategies for related disorders.

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Disclosures

The authors declare that they have no competing financial interests.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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