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INVITED REVIEW

Male Endocrinology

Human androgen deficiency: insights gained from androgen receptor knockout mouse models

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The mechanism of androgen action is complex. Recently, significant advances have been made into our understanding of how androgens act via the androgen receptor (AR) through the use of genetically modified mouse models. A number of global and tissue-specific AR knockout (ARKO) models have been generated using the Cre-loxP system which allows tissue- and/or cell-specific deletion. These ARKO models have examined a number of sites of androgen action including the cardiovascular system, the immune and hemopoetic system, bone, muscle, adipose tissue, the prostate and the brain. This review focuses on the insights that have been gained into human androgen deficiency through the use of ARKO mouse models at each of these sites of action, and highlights the strengths and limitations of these Cre-loxP mouse models that should be considered to ensure accurate interpretation of the phenotype.

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INTRODUCTION

The use of rodent models for investigating the function of androgens allows clinically important questions to be asked that are difficult or impossible to address in humans. A variety of mouse models have contributed significantly to this research. These approaches include (1) removing testosterone by orchidectomy; (2) blocking testosterone action by utilizing androgen or gonadotropin antagonists; and (3) genetically modified models targeting androgen synthesis or androgen action. This review will focus on genetically modified models ablating androgen action.

The ability to delete genes globally in the entire animal or in a tissue- and/or time-specific manner via the Cre-loxP system in mice has been utilized in endocrinology research since the technology first became available. The precise and tight regulation of this approach enables the investigation of complex pathways in heterogeneous tissues. In androgen research, the most widely used models have targeted the androgen receptor (AR). The five genetically modified AR mouse lines now available have been utilized to investigate tissue-specific effects of the AR in multiple tissues including the cardiovascular system, the immune and hemopoetic systems, bone, muscle, adipose tissue, the prostate and the brain. The standard experimental paradigm deletes the AR globally or in a known target tissue in live mice and compares these with appropriate wildtype (WT) controls. This approach clearly defines the function of the AR in the tissue of interest.

Different physiological effects of removing testosterone and its receptor, the AR

Testosterone is metabolized to 5α-dihydrotestosterone (DHT), and both of these hormones act via the AR. Testosterone is also metabolized to 17β-estradiol which acts via the estrogen receptors (ER). Thus, one would expect that removal of the testes in a male would eliminate the effects of testosterone and DHT acting via the AR and also substantially reduce estradiol acting via the ER. By comparison, global inactivation of the AR targets androgen actions directly. Orchidectomy in rodents reduces circulating testosterone levels close to zero, as rodents do not produce adrenal androgens[1] but may have some local synthesis. Thus, some physiological processes abolished by deleting the AR may not be entirely abolished in models utilizing orchidectomy. Similarly pharmacological AR antagonists are rarely 100% effective. Finally, orchidectomy acts from the time of the procedure, whereas Cre-loxP mediated deletion of the AR in the current models causes deletion *in utero*.

DNA binding-dependent and non-DNA binding-dependent actions of the AR

Ligand-bound AR modulates the transcription of target genes via the classical or DNA-binding pathway.[2] The androgen/AR complex can also signal through non-DNA binding-dependent (or non-genomic) pathways[3] (**Figure 1**). These include rapid activation of second messenger pathways, such as ERK, Akt and MAPK, occurring within seconds to minutes of androgen treatment. This is too rapid to involve gene transcription.[3-6] Indirect gene transrepression can also occur, by the AR binding and sequestering transcription factors, such as activating protein (AP)-1, that are normally required to upregulate target gene expression in the absence of the AR binding to DNA (e.g. *Ngfr*[7] and *Mmp-13*[8]).

The physiological relevance of non-DNA binding-dependent AR actions remains controversial, primarily because of the lack of *in vivo* studies.[9] However, effects including rapid coronary vasodilation[10]

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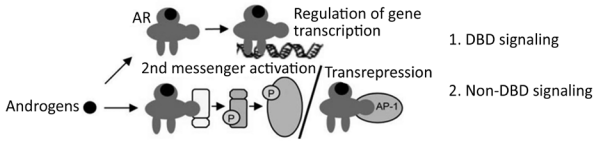

Figure 1: Signaling pathways of the androgen receptor (AR). Androgens bind to the AR and (1) regulate gene transcription via DNA binding-dependent (DBD) signaling or (2) activate second messenger pathways or transrepression through non-DBD signaling.

and oocyte maturation[6,11] have been documented, and there is now significant interest in non-DNA binding-dependent actions of the ER and glucocorticoid receptor, [12] which are functionally similar to the AR.

THE CRE-LOXP SYSTEM

The Cre-loxP system is the most flexible of the knockout (KO) systems in use. In theory, by using specific promotor constructs, deletion of the AR can be controlled in a tissue- and/or time-specific manner. The Cre-loxP system utilizes two genetically modified mouse lines. The Cre line contains the Cre recombinase enzyme, the expression of which is driven by a tissue-specific promoter (**Figure 2**). The genome of the loxP or "floxed" line contains two loxP sequences flanking the region of the target gene to be deleted. The loxP sites are inserted in such a way as to not modify the function of the target gene. When the two lines are crossed, the Cre enzyme recognizes the two loxP sequences and deletes the sequence between the two sites leaving a single loxP sequence only in tissues where the Cre is expressed. LoxP sites are introduced into mice by homologous recombination in embryonic stem (ES) cells and the Cre mice are generated using standard transgenic technology. The power of this system derives from the ability to use a large variety of available promoter sequences to target expression of the Cre recombinase in a tissue- or cell-specific and/or in a time-specific manner. Global KO animals can be produced by using a ubiquitously expressed Cre such as cytomegalovirus (CMV)-Cre.

To date, five different floxed AR mouse lines in which pairs of loxP sites have been inserted into the *AR* gene have been generated:

1. AR^{L2}: exon 1 deletion; frameshift mutation; Kato laboratory[13]
2. $Ar^{flox\ (ex1\text{-}neo)}$: exon 1 deletion; frameshift mutation; Braun laboratory[14]
3. fAR: exon 2 deletion; frameshift mutation; Chang laboratory[15]
4. AR^{flox}: exon 2 deletion; frameshift mutation; Verhoeven laboratory[16]
5. AR^{lox}: exon 3 deletion; in-frame deletion; Zajac laboratory.[17]

All of the global ARKO models generated using the AR floxed mice listed above (1,3,4), with the exception of our model (5), are AR-null as they have a frameshift mutation resulting in no AR expression. Our global ARKO model has an in-frame deletion of exon 3 (deletion of the second zinc finger of the DNA binding domain)[17] and retains non-DNA binding-dependent actions in all tissues as the mutant AR protein is still expressed.[18] This model was generated on a controlled C57BL/6 background and is now referred to as the $AR^{\Delta ZF2}$ model. With regards to the genetic background of the other global models, the AR-null ARKO model generated by the Kato laboratory, using their exon 1 floxed AR mouse line, has a mixed C57BL/6 and CD-1 genetic background.[13] The AR-null ARKO model by the Chang laboratory, generated using their exon 2 floxed AR line, has a mixed C57BL/6 and 129SVE background.[15] The third AR-null ARKO model generated by the Verhoeven laboratory using

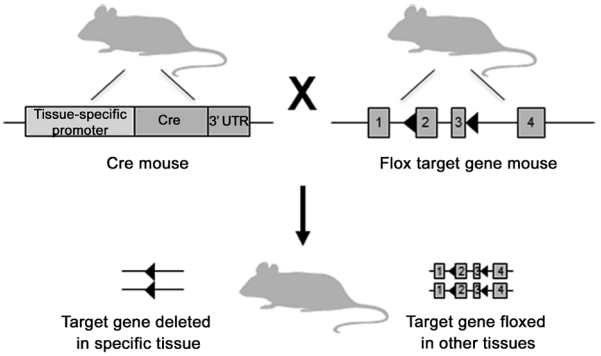

Figure 2: Generation of tissue/cell-specific knockout mice using the Cre-loxP system. In the Cre mouse line, the expression of Cre is under the control of a tissue/cell-specific promoter. The floxed target gene mouse line contains loxP sites (◀) flanking the region of the target gene to be deleted. When the two mouse lines are bred together, the Cre enzyme recognizes the loxP sites and deletes the intervening DNA sequence only in tissues/cells where the Cre is expressed. The target gene remains floxed and theoretically functional, in all other tissues.

their exon 2 floxed AR line is maintained on a C57BL/6 background.[16] Differences in the AR deletion and genetic backgrounds of these different models therefore, may account for the phenotypic variations observed between the models as discussed in further detail below.

Limitations of Cre-loxP models

There are a number of limitations of the Cre-loxP system, which may not be apparent to those outside the field. These have been reviewed extensively by ourselves and others[19-21] and as such will not be discussed in detail in this review, but rather we will highlight the more common limitations that researchers should be aware of when interpreting the phenotype of such models. The position of the loxP sites and neomycin (neo) selection cassette (required for selection of ES cells) within the target *AR* gene in the floxed line can alter expression levels. Holdcraft and Braun[14] reported that their $Ar^{flox\ (ex1\text{-}neo)}$ mice have a hypomorphic phenotype due to the presence of a selection cassette. Similarly, we observed a phenotype of mild hyperandrogenization in our AR^{lox} male mice which retained the neo selection cassette as evidenced by changes in the mass of a number of androgen-responsive tissues.[22] More recently, we have generated a neo-negative AR^{lox} line, where the neo cassette has been removed. These mice exhibit normal tissue mass including kidney, heart, testis and seminal vesicle (unpublished data).

Another major factor to be considered is both the level and tissue-specificity of Cre expression. Cre may not be expressed at sufficient levels to cause 100% deletion of the target gene and therefore no phenotype may be apparent due to residual expression of the target gene. It is therefore important to characterize the degree of Cre-mediated deletion of the target gene. A lack of discernable phenotype may also be attributed either to the AR having no function in the target tissue or that it is not important in basal conditions, but rather in times of stress. Distinguishing between these possibilities requires careful phenotyping of the model and can be at times difficult. An additional problem is non-specific deletion of the target sequence in tissues outside the tissue of interest. Promoters are commonly not 100% tissue- and/or time-specific. Often published studies focus on the tissue of interest without reporting whether non-specific deletion is occurring in other tissues. Expression of Cre and deletion of the AR in other unexpected tissues may influence the conclusions which can be drawn from the data.

The nature of the Cre-loxP system typically results in mice of a mixed genetic background being generated. Since the phenotype of

mice can differ between different genetic backgrounds (including reproductive traits such as litter size and sperm production, metabolic characteristics, bone density and kidney and adrenal weight indexes), the results can often be confounded by the effect of genetic background.[19] It is therefore important to backcross Cre-loxP mice to a homogenous background to ensure accurate interpretation of the phenotype.

Other limitations of rodent models

The physiology of rodent models is not identical to that of humans. Nonetheless, there are very substantial similarities. The mouse genome is 85% similar to the human genome and many features of the mouse genotype are almost identical to those in humans. There are some significant differences in the physiology of androgens between mice and men, for example, the lack of production of androgens in the adrenal glands in mice and the lower degree of protein binding of circulating testosterone.[19]

In relation to bone studies, one of the most obvious differences between humans and mice is of course posture and the resulting biomechanical forces within bone. Unlike humans, linear growth also continues in rodents with no epiphyseal closure, but does reduce to minimal levels by 4 months of age.[23]

ANDROGEN DEFICIENCY AND THE CARDIOVASCULAR SYSTEM

The role of androgens in the cardiovascular system in humans is complex and confusing as described by Bu B Yeap elsewhere in this issue. Human males have a significantly higher risk of cardiovascular disease than females, but aging males with lower levels of testosterone have higher cardiovascular mortality than males with higher testosterone. Furthermore males with cardiac disease, particularly with ventricular failure, have lower levels of testosterone. A recent trial of testosterone supplementation in males caused an increase in cardiovascular events.[24] Thus, the role of testosterone acting on the cardiovascular system may well be important, but remains very poorly understood.

Mouse models

There is only a small amount of data on the cardiovascular system obtained from ARKO mouse models. One global ARKO model has been investigated in terms of cardiovascular function, demonstrating low levels of testosterone but normal levels of estradiol.[25] These ARKO mice displayed small hearts in relation to body weight with impaired contraction. In ARKO mice treated with angiotensin II, there was impairment of adaptive concentric cardiac hypertrophy and left ventricular function, as well as a reduction in the size of cardiomyocytes. Angiotensin-induced cardiac fibrosis was also enhanced in the ARKO mice.[25] These effects on cardiac hypertrophy may be mediated by ERK1/2 and ERK5. This data suggests that the AR may be involved in cardiac growth, hypertrophy and fibrosis. As these mice are global ARKOs, generation of cardiac-specific ARKOs are required in order to ascertain definitively whether these effects are generated directly via cardiac muscle.

Relevance to human physiology

The identification of a role of the AR in specifically regulating cardiac muscle, fibrosis and the cardiovascular system in mice could identify new therapeutic targets for treating both cardiac failure and ischemic heart disease in humans.

ANDROGEN DEFICIENCY AND THE IMMUNE AND HEMOPOETIC SYSTEMS

Females have an increased incidence of autoimmune disease compared to men.[26] On the other hand, males are more likely to develop sepsis and multiorgan failure after traumatic hemorrhagic shock and thermal injury, partly because of immune suppression.[27] Thus, it is likely that the AR is involved in the regulation of immune function.

Mouse models

A variety of models have been generated with a diverse array of results, some of which are discussed here.

A number of cell lineages express and are regulated by the AR. Neutrophil count has been shown to be reduced by 90% in global ARKOs,[28] but bone marrow mesenchymal stem cells from ARKO animals demonstrate a greater ability to self-renew.[29] This self-renewal occurred over several generations and these cells were shown to be able to differentiate into osteoblasts and adipocytes.

Castration of animals accelerates wound healing[30] and ARKO mice have accelerated wound healing.[31] Furthermore, transplantation of ARKO bone marrow into irradiated WT mice also enhances wound healing. A Lyz-Cre mouse line targeting fibroblasts[32] was utilized to generate myeloid-specific ARKO mice, which also exhibited accelerated wound healing.

In mice, the AR may function to regulate both innate and adaptive immunity where the AR may exert suppressive effects on the development and activation of both T and B cells.[28] In a recent paper by Lai *et al*.,[33] AR deletion led to increased bone marrow transplant grafting efficiency.

Although the most obvious effect of androgens on the hemopoetic system in humans is the higher level of hemoglobin in males than females, this has not been investigated in ARKO mice.

Relevance to human physiology

Clear differences in the immune response in these ARKO animals may suggest similar effects in humans which may help explain the sexually dimorphic nature of immune responses and the incidence of autoimmune disease. Further work is warranted.

ANDROGEN DEFICIENCY AND BONE

Fractures during aging are a major public health problem (as discussed by Laurent *et al*. elsewhere in this issue). One in two women and one in three men over the age of 60 experience a fragility fracture. The role of sex-steroids in the pathogenesis of osteoporosis has been extensively studied in women, but less so in men. Contributing to this disparity is the fact that the decline in testosterone levels in men does not occur as rapidly as the decline in estrogen levels in women after menopause. Androgens contribute to reducing the risk of fracture in elderly men by two known mechanisms:

1. Androgens play a significant role in determining peak bone mass or maximum bone density as they are essential for skeletal growth and bone accrual during puberty[34]
2. Androgens maintain bone in postpubertal males,[35] determining both the size and strength of adult bone.[36]

The mechanisms by which androgens exert these effects on bone are poorly understood. The relative importance of androgen action on bone mediated directly via the AR or indirectly following aromatization to estradiol and acting via the ER[37] remains to be determined. The anabolic actions of androgens, together with their potential but less well defined anticatabolic action, makes androgen physiology a key candidate for understanding the bone fragility of aging men. In order for such androgenic activities to be exploited clinically, it is necessary to elucidate the cellular mechanisms by which androgens exert their effects in bone.

Mouse models

Insight gained through the use of ARKO models has advanced our understanding of the mechanism of androgen action on bone.

We and others have shown that global deletion of the AR in males results in bones of reduced size, thickness and volume compared to controls[15,38-40] (**Figure 3**), indicating these key actions of androgens require the AR. In female AR$^{\Delta ZF2}$s, we also observed a small, but significant decrease in bone size but this was characterized by decreases in periosteal and medullary circumference with no change in trabecular bone volume, despite reduced trabecular number and increased trabecular thickness, indicative of increased bone turnover.[38] This was the first report in females of a possible role of androgen action via the AR, in addition to the well-characterized actions via estradiol to regulate cortical bone growth.

Androgen action in osteoblasts and osteocytes

There is convincing evidence indicating that the effects of androgens on bone are mediated, at least in part, by the AR expressed in osteoblasts and osteocytes.[41,42] Transgenic mice overexpressing the AR specifically in proliferating osteoblasts under the control of the 3.6 kb type 1a1 collagen (Col1a1) promoter, including those located at the periosteum, have larger bones due to increased periosteal mineral apposition.[43] In contrast, overexpression of the AR in mineralizing osteoblasts under the control of the 2.3 kb Col1a1 promoter has no effect on bone size.[44] Both AR transgenic models do however, have a common phenotype of increased trabecular bone volume as a result of reduced bone turnover.[43,44] Consistent with these findings in AR overexpressing mice, we have shown that deletion of the AR specifically in osteoblasts and osteocytes from either the i) mature or ii) mineralization stage of osteoblast maturation, generated by breeding our floxed AR mice[17] with i) Col2.3Cre[45] or ii) osteocalcin-Cre mice,[46] has the opposite effect of trabecular bone loss due to increased bone resorption.[47,48] This is evidenced by reduction in trabecular bone volume and number in the osteoblast-AR$^{\Delta ZF2}$ models, compared to controls.[47,48] Reduction in cortical bone and a dysregulation of the bone matrix synthesis and mineralization processes were also observed in mice lacking the AR in mineralizing osteoblasts (generated using osteocalcin-Cre), identifying an important role of the AR in regulating bone resorption and mineralization, particularly during pubertal growth when rapid bone formation is required. Furthermore, Sinnesael *et al*.,[49] have shown that inactivation of the AR specifically in terminally differentiated osteocytes, driven by the dentin matrix protein 1 promoter decreased trabecular bone, but did not impair its response to mechanical loading.

Taken together, these studies suggest that androgen action via the AR on osteoblasts is dependent on the stage of osteoblast maturation with AR activation in mineralizing osteoblasts and osteocytes inhibiting bone resorption within cortical and trabecular bone, while activation of the AR in proliferating osteoblasts mediates an anabolic effect on cortical bone at the periosteum.

Further study is required to identify the mediators of these androgen effects in bone. As an initial step we have employed targeted gene expression and microarray approaches[50] and identified a number of osteoblast and osteoclast genes upregulated in the bones of osteoblast-AR$^{\Delta ZF2}$s, consistent with the increased bone turnover observed in these mice.[48] Of significant interest, we have also identified genes involved in carbohydrate and fat metabolism and growth and development as potential targets of androgen action via the AR in mineralizing osteoblasts.[50]

Androgen action on osteoclasts

A role for androgens regulating bone resorption directly via the AR on osteoclasts remains controversial. Conflicting findings of no effect[51,52] or inhibition of osteoclast-like cell formation and bone resorption following androgen treatment *in vitro*[53-55] have been reported. Interpretation of these findings is difficult due to the many different models of osteoclastogenesis used. In addition, testosterone which can be aromatized to estradiol, a potent inhibitor of bone resorption, was used to treat the osteoclast-like cells. The osteoclastogenic factor, receptor activator of nuclear factor κβ ligand (RANKL), has been shown to be upregulated in ARKO osteoblasts.[39] However, the obvious means to obtain definitive proof as to whether androgens act directly *via* the AR in osteoclasts to regulate bone resorption is the generation of a mouse model in which the AR is deleted only in osteoclasts. Only one such osteoclast-specific ARKO mouse model has been generated to date by Sato *et al*.[13] in 2004; however, these data have only been presented in abstract form and are yet to be published. In this study, osteoclast-ARKO mice were generated by breeding floxed AR mice with a cathepsin K-Cre knockin mouse line, where the endogenous cathepsin K gene was disrupted by insertion of the Cre transgene. Osteoclast-ARKOs displayed an increased number of osteoclasts within the lumbar spine, suggesting that androgens exert an inhibitory action on osteoclasts directly via the AR. Further studies are required to confirm these findings.

Non-genomic actions of the AR in bone

To date, evidence for a role of the non-DNA binding-dependent signaling pathway of the AR in regulating bone cell metabolism has been limited to *in vitro* studies.[4,5] Recently, we have provided evidence using our AR$^{\Delta ZF2}$ mouse model to support a physiological role of the non-DNA binding-dependent AR pathway in regulating bone *in vivo.* This was achieved by treating AR$^{\Delta ZF2}$s with the non-aromatizable androgen, DHT. DHT treatment suppressed the outer growth of cortical bone in AR$^{\Delta ZF2}$s, with a 6% reduction in periosteal circumference and a 7% decrease in medullary circumference versus untreated AR$^{\Delta ZF2}$s. These effects must have arisen via the non-DNA binding-dependent actions of the AR, as the DNA-binding dependent actions are absent in AR$^{\Delta ZF2}$s. The predominant action of androgens in cortical bone is thought to be stimulation of bone growth by increasing periosteal bone apposition,[56] presumably *via* the dominant DNA binding-dependent AR pathway. The fact that DHT inhibited periosteal bone growth in

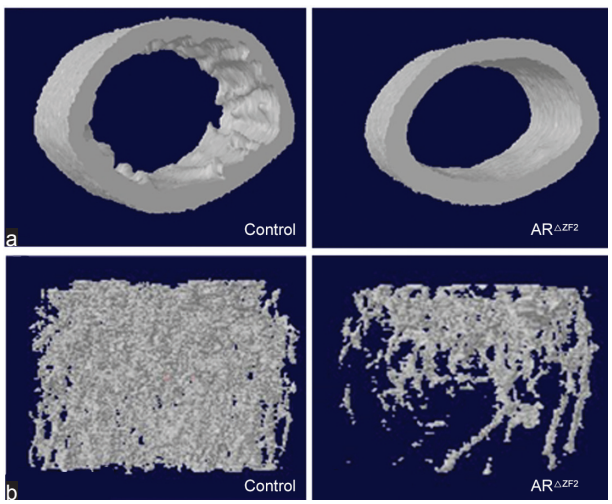

Figure 3: 3D micro-computed tomography (μCT) images showing that removal of the androgen receptor (AR) in male AR$^{\Delta ZF2}$ mice results in smaller and thinner bones of decreased density. **(a)** Cortical and **(b)** Trabecular bone.

$AR^{\Delta ZF2}$ males suggests this opposing action is mediated via a non-DNA binding-dependent AR pathway, similar to the opposing actions of the DNA binding-dependent and non-DNA binding-dependent pathways observed for the ERα.[57,58] The reduction of bone formation at the endocortical surface, reflected by decreased medullary circumference, is consistent with previous observations of inhibited endocortical bone formation in a mouse line with osteoblast-targeted overexpression of the AR,[44] suggesting that both DNA binding-dependent and non-DNA binding-dependent pathways of the AR also regulate endocortical bone formation. The opposing and balancing DNA binding-dependent and non-DNA binding-dependent actions of the AR on cortical bone growth may provide a fine tuning mechanism by which bones can adapt and respond to changes in mechanical load during growth and development.[18]

Relevance to human physiology

Understanding the precise mechanisms by which androgens exert their actions on bone will provide significant information on their role in optimizing peak bone mineral status during growth and the emergence of bone fragility during aging. This information has the potential to provide new avenues for the use of therapeutic agents, such as selective AR modulators, which might target the AR within specific bone cell types to increase bone size and volume. This is particularly important as current forms of androgen therapy have side effects in aging men and cannot be used in women.

ANDROGEN DEFICIENCY AND MUSCLE

In men, androgens are required during growth and development to achieve normal peak muscle mass and strength as discussed by Matthew DL O'Connell and Frederick CW Wu elsewhere in this issue. Men have larger and stronger muscles than women and hypogonadal men have reduced lean body mass.[59] Androgen treatment increases lean body mass and muscle strength in hypogonadal men[59,60] as well as in men with normal androgen levels.[61,62] Randomized controlled studies have demonstrated a dose-dependent response to administered testosterone in both young and elderly men, with increases in muscle size and strength associated with fiber hypertrophy.[63,64] Testosterone treatment in frail elderly men with low-borderline testosterone levels has been shown to prevent loss of limb strength and improve body composition.[65] Androgens are required to maintain muscle mass in men, with androgen withdrawal causing muscle atrophy. Loss of muscle mass in adult men following androgen withdrawal has been demonstrated in both normal men and men with prostate cancer undergoing androgen deprivation therapy.[66-68] The decline in testosterone levels with age in males may be one mechanism contributing to the age-related loss of muscle mass that occurs in elderly frail men.

Mouse models

Using our global $AR^{\Delta ZF2}$ model, we have shown that the AR is required for development of normal muscle mass and strength in males.[69] Hind-limb muscle mass is reduced by 15%–22% in our global $AR^{\Delta ZF2}$ males compared to WT males, and the fast-twitch extensor digitorum longus muscle from $AR^{\Delta ZF2}$ males has reduced contractile strength;[69] whereas, the slow-twitch soleus has increased fatigue resistance. Other AR-null ARKO models[70,71] demonstrate a milder muscle phenotype compared to the global $AR^{\Delta ZF2}$ model.

Two muscle-specific ARKO (mARKO) mouse models have been reported both with AR-null alleles that delete all AR-mediated actions. A myofiber-specific AR-null mARKO mouse model, generated using MCK-Cre, has little change in the mass of hind-limb muscles, but a reduction in the mass of the highly androgen-dependent levator ani (LA) muscle.[72] The muscle phenotype of these myofiber-specific mARKO mice is also similar to this group's global AR-null mice.[71] A muscle-specific mARKO mouse line, generated using human skeletal actin-Cre, with deletion of the AR in both myoblasts and myofibers also has no reduction in hind-limb muscle mass, but reduced LA muscle mass.[73] Our group has recently generated a myofiber-specific $mAR^{\Delta ZF2}$ mouse model, generated using MCK-Cre (unpublished data). LA mass in our $mAR^{\Delta ZF2}$ male mice is reduced by 53%, but there is small to no effect on hind-limb muscle mass which suggests androgens act earlier in the muscle cell lineage than mature myofibers or through other tissues (**Figure 4**). The phenotypic differences in the models may arise because of different methodologies used to assess muscle mass, mixed genetic backgrounds of the AR-null models or the fact that our model $AR^{\Delta ZF2}$ has deletion of genomic actions, but retains potential non-genomic actions as the mutant AR protein is expressed.

Relevance to human physiology

Despite their widespread illicit use to build muscle strength and mass, androgens are not widely used therapeutically to increase skeletal muscle mass and strength because of negative side effects. Understanding the mechanism of the powerful anabolic effect of androgens on muscle is essential for developing better and more specific therapies to strengthen muscle in chronic disease and other conditions of muscle wasting.

ANDROGEN DEFICIENCY AND GLUCOSE REGULATION, METABOLISM AND OBESITY

Obesity is associated with adverse metabolic consequences, including dyslipidemia and type 2 diabetes.[74] Low testosterone levels are associated with insulin resistance in men with obesity and type 2 diabetes,[75,76] and insulin resistance independent of obesity in nondiabetic men[77] as discussed by Carolyn A Allan as well as Mathis Grossmann and his colleagues elsewhere in this issue. The cause and effect relationship of this association remains uncertain. It is still not clear if low testosterone levels are a general cause of obesity and insulin resistance in eugonadal men or a consequence of obesity.[78,79] Testosterone supplementation in hypogonadal men has been shown to decrease abdominal fat mass,[60] and improve insulin sensitivity in some studies,[80] but not others.[81]

Mouse models

A metabolic phenotype has been described in three of the global ARKO models. Our global $AR^{\Delta ZF2}$ male mice have increased infrarenal and subcutaneous fat pad mass,[82] despite a reduction of total body mass of ~12% compared to WT littermates.[69] Global $AR^{\Delta ZF2}$ males have normal insulin sensitivity at all ages, demonstrating that DNA binding-dependent actions of the AR regulate fat mass but have no measurable effect on insulin sensitivity. The increased fat mass observed is likely to be due in part to their decrease in voluntary

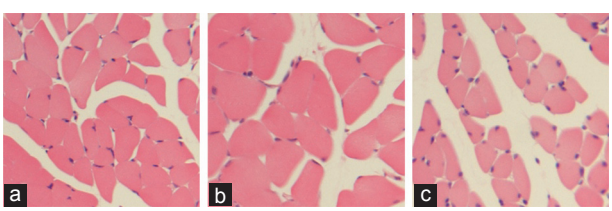

Figure 4: Cross-sectional area of levator ani (LA) muscle from **(a)** Wildtype (WT), **(b)** Floxed androgen receptor (AR) and **(c)** $mAR^{\Delta ZF2}$ male mice. LA muscle mass is reduced by 53% in $mAR^{\Delta ZF2}$ males compared to WT males ($P < 0.001$).

physical activity.[82] An adipose phenotype has been reported in two global AR-null ARKO models,[83,84] that differ significantly from our model. Both these models have a late-onset obesity phenotype, with body weight of ARKO males increased by 20%-40% versus control males. This appears from age 9 weeks and is most apparent by 20 weeks of age. Fat pad mass is also increased in these mice, associated with hyperinsulinemia, increased muscle triglyceride content, and increased levels of serum leptin[83] and adiponectin.[84] The differences between our results and previous reports may be because our $AR^{\Delta ZF2}$ mice are strictly controlled on a C57BL/6 genetic background or alternatively, may reflect a physiological role for non-genomic actions of the AR in regulation of fat metabolism.

An adipose tissue-specific ARKO model, generated using adipocyte specific fatty acid binding 4 protein (aP2)-Cre, demonstrates a phenotype of hyperleptinemia but no leptin resistance.[85] Interestingly, these mice are not obese, with normal body weight, a normal adiposity index and normal adipocyte size. The authors concluded that the AR in adipose tissue has a differential role in energy balance. More recently, another adipose tissue-specific ARKO model was reported, using the same Cre but a different floxed AR line, and showed hyperinsulinemia in the absence of obesity.[86] These mice, when placed on a high fat diet, had increased susceptibility to visceral obesity and hyperglycemia and impaired insulin secretion. Interestingly, the AR-null mARKO model generated by the Verhoeven group, demonstrated a reduction in intra-abdominal fat associated with a reduction in muscle mass.[72] The authors suggest this phenotype was due to the switch to a slower, oxidative fiber type in muscle, although metabolic rate and insulin sensitivity were not assessed. Recently, neuronal ARKO mice were generated using synapsin I-Cre and found to have a phenotype of reduced insulin sensitivity associated with increased visceral obesity, and increased serum triglycerides and free fatty acids.[87] Deletion of the AR in the brain focused attention on regulation of insulin sensitivity via the hypothalamus.[87] In this model, deletion of the AR caused hypothalamic insulin resistance with secondary effects to increase hepatic insulin resistance, lipid accumulation and increase in visceral adipose tissue. The mice also had a greater weight response to a high fat diet.

The global ARKO models suggest that fat mass is regulated by the AR; however, the lack of an obese phenotype in the adipose tissue-specific ARKO model on a normal diet and the presence of a metabolic phenotype in muscle- and neuronal-specific ARKO models suggest that this regulation is occurring through AR actions in tissues other than fat, including skeletal muscle and the brain/central nervous system (CNS).

Relevance to human physiology

Understanding the mechanisms by which androgens and the AR are involved in the regulation of metabolism, energy regulation and weight control will provide significant clinically relevant information. These interrelated metabolic regulatory cascades are particularly complex to dissect and animal models are likely to provide answers which can then be tested in clinical settings. This will lead to new therapeutic approaches. Actions of androgens in the brain may contribute to the insulin resistance seen following androgen deprivation therapy for prostate cancer,[68,88] as well as the increase in visceral adipose tissue. Activation of the AR in the brain may be a target for therapy in the future.

ANDROGEN DEFICIENCY AND THE PROSTATE

The role of androgens in regulating prostate structure and function is not straight forward as discussed by Lori A Cooper and Stephanie T Page elsewhere in this issue. Androgens are required for prostate development. However, there is much recent data in relation to the development of prostate cancer and the response of prostate cancer to treatment in which the role of androgens acting via the AR is unclear. Local synthesis of testosterone acting via the AR or through aromatization to estrogen clearly has a role in regulating prostate function.

Mouse models

Global ARKO models show no prostate development,[13,15-17] however, the prostate does develop in cell-specific prostate ARKO models. Models targeting the epithelium or the stroma of the prostate have been studied to understand the interplay between these two cell types.

Prostrate epithelial ARKO (PEARKO) mice, generated using probasin-Cre, in which the DNA binding activity of the AR is deleted in the epithelium of the prostate, the epididymis and the vas deferens, demonstrates decreased anterior and dorsolateral lobe weight, as well as increased epithelial proliferation and abnormal epithelial clustering, particularly in the anterior lobe.[89] There is clear evidence in the PEARKO model for increased sensitivity to estradiol, due to an increase of ERα expression in the dorsolateral prostate epithelial cells, suggesting that ERα expression in prostate epithelial cells is regulated by local epithelial AR-dependent mechanisms.[90] Pes-ARKO mice, generated utilizing a different form of the probasin promoter resulting in AR deletion in the ventral prostate and the dorsolateral prostate, demonstrate a phenotype of increased ventral prostate size with increased epithelial proliferation in the ventral prostate and dorsolateral prostate.[91] The authors concluded that this model supports the hypothesis that the AR regulates growth by suppressing epithelial proliferation.

Mouse models targeting the stroma include the smooth muscle (SM) and fibroblast ARKO models. Two SM models have been reported. The peritubular myoid-specific ARKO (PTM-ARKO), generated using SM myosin heavy chain-Cre resulting in AR deletion in SM cells in all prostate lobes, demonstrates decreased prostate weight, hyperplasia, inflammation and increased estradiol sensitivity.[92] In contrast, the SM-ARKO, generated using transgelin-Cre which has highest expression in the anterior prostate, has no change in gross appearance of the prostate but has loss of infolding structures into the lumen (mainly in the anterior prostate), decreased epithelial proliferation and decreased insulin-like growth factor-1 (IGF1) expression levels.[93] The recently described FSP-ARKO model (fibroblast-specific protein 1-Cre), by the same group, also shows no gross structural change but histological examination demonstrates decreased epithelial proliferation, increased apoptosis and reduced collagen deposition.[94] These mice also show decreased expression of a number of growth factors including IGF1.

Recently, double stromal fibromuscular-specific ARKO (dARKO) mice were reported.[95] This model was generated using two Cre mouse lines, fibroblast-specific protein 1-Cre and transgelin-Cre mice; deleting the AR in both stromal and SM cells. dARKO mice showed reduced prostate size due to decreased anterior prostate size and abnormal branching morphogenesis, with partial loss of glandular infolding structure, decreased proliferation and increased apoptosis of the epithelium in the anterior prostate.[95]

Relevance to human physiology

Identification of the effects of androgens acting on specific cells in the prostate, either directly via the AR or through ligand independent AR action, may lead to agents which target the AR in a clinically cell-specific manner. This may have major implications for the development of innovative treatments for prostate cancer.

ANDROGEN DEFICIENCY AND THE BRAIN

Whilst there is a large body of data that sexual function and reproduction in males is dependent on androgen effects in the CNS,[96] the mechanism by which androgens exert these effects are poorly understood. Similarly, research on the role of androgens in the regulation of cognitive function, mood and metabolic control has been complicated by cultural and political factors and factors related to upbringing.[97] Research focusing on spatial ability has produced the most consistent and reproducible findings suggesting that men perform some tasks such as mental rotation, maze completion and real-world navigation more quickly and accurately than women.[98,99] In addition, a number of studies demonstrate a link between circulating levels of testosterone with sexual differences in spatial ability.[100,101] All of this data has been culturally or socially determined, therefore it remains to be ascertained how much of these gender differences in humans reflect biological differences versus cultural effects.

Mouse models

AR expression is widespread throughout the brain in both male and female mice, but recent data demonstrates that AR expression in the suprachiasmatic nucleus of the hypothalamus is higher in males than in females.[102] Available mouse models include various global ARKOs, as well as the brain-specific nestin-Cre ARKO[103] and synapsin I-Cre ARKO[87] models. Global deletion of AR using CMV-Cre caused abolition of male sexual and aggressive behaviors.[13] These mice displayed no male-specific sexual behavior at all (mounting, intromission and ejaculation). Global ARKO mice however, have female external genitalia making interpretation of much of the data complex. There was no effect on female behavior. The two brain-specific models circumvent this problem. They target neurons but are likely to have different patterns of KO. These models have been used to address the question of whether the actions of androgens in the brain are mediated directly by the AR or indirectly via aromatization to estradiol via the ER. Male mice with both forms of ER deleted displayed a complete absence of male mating and territorial behavior,[103] despite the fact that testosterone levels were normal; therefore, suggesting testosterone was acting via its conversion to estradiol and action via the ER. Nestin-Cre ARKO mice still displayed male sexual and territorial behavior, but less frequently than control mice. The authors conclude that testosterone in the neonatal period acts via the ER in the brain to cause appropriate male mating and aggression circuits to form. They postulate that testosterone in adult mice acting via the AR regulates the frequency of mating behavior and aggressive behavior. These ARKO mice display normal reproductive mating behavior and aggressive behavior, but mate less frequently and spend less time fighting. Thus, the AR is not essential for masculinization of mating and aggressive behavior. AR amplifies these behaviors and regulates their nature and frequency. However, the nestin-Cre ARKO model may have the same limitations of most Cre-loxP animals in that it is difficult to be certain that the degree of deletion of the AR is significant in all CNS neurons and there is no clear data on differences in deletion intensity in various types of neurons. At this stage, there is little research on cognitive function or mood using ARKO animals. Metabolic effects mediated via the brain are discussed above in the obesity section.

Relevance to human physiology

The data if applied to humans may suggest that in males with androgen deficiency, the decrease in sexual behavior and libido is mediated by testosterone acting via the AR rather than via aromatization to estradiol. The immense field of the effect of androgens on behavior, cognitive function memory and mood remains open for study.

CONCLUSION

A great deal has been learned about the actions of androgens via the AR in a diverse range of tissues through the generation of genetically modified mouse models. The similarity of the genomes and physiology of mice and humans make these models immensely useful in helping to understand the specific functions of androgens acting via the AR in different tissues. Cre-loxP technology is a rapidly developing field, with the generation of new tissue-/cell-specific Cre mouse lines as well as major advances in the development of improved inducible Cre mouse lines which enable the expression of Cre to be switched on or off at different stages of development. The use of these improved mouse models will ensure the significant progress of our understanding into the mechanisms of AR action underlying the pathology of human androgen deficiency.

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COMPETING INTERESTS

The authors have nothing to declare.

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