

Androgen receptor (AR) in cardiovascular diseases

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Abstract

Cardiovascular diseases (CVDs) are still the highest leading cause of death worldwide. Several risk factors have been linked to CVDs, including smoking, diabetes, hyperlipidemia, and gender among others. Sex hormones, especially the androgen and its receptor, androgen receptor (AR), have been linked to many diseases with a clear gender difference. Here, we summarize the effects of androgen/AR on CVDs, including hypertension, stroke, atherosclerosis, abdominal aortic aneurysm (AAA), myocardial hypertrophy, and heart failure, as well as the metabolic syndrome/diabetes and their impacts on CVDs. Androgen/AR signaling exacerbates hypertension, and anti-androgens may suppress hypertension. Androgen/AR signaling plays dual roles in strokes, depending on different kinds of factors; however, generally males have a higher incidence of strokes than females. Androgen and AR differentially modulate atherosclerosis. Androgen deficiency causes elevated lipid accumulation to enhance atherosclerosis; however, targeting AR in selective cells without altering serum androgen levels would suppress atherosclerosis progression. Androgen/AR signaling is crucial in AAA development and progression, and targeting androgen/AR profoundly restricts AAA progression. Men have increased cardiac hypertrophy compared with age-matched women that may be due to androgens. Finally, androgen/AR plays important roles in contributing to obesity and insulin/leptin resistance to increase the metabolic syndrome.

Key Words

- ▶ gender difference
- ▶ androgen
- ▶ castration
- ▶ male hormone

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Introduction

Globally, cardiovascular diseases (CVDs) are still the highest cause of death worldwide in developed countries and developing countries (Gaziano *et al.* 2010). Several risk factors, including smoking, diabetes, and hyperlipidemia, play critical roles in the occurrence of CVDs (Zimmerman 2012). The gender difference is another key risk factor affecting CVDs (Banos *et al.* 2011).

Among the factors associated with the gender difference, sex hormones, including estrogens

and androgens, are two key factors that have been studied extensively (Simon 2001). Postmenopausal women have decreased estrogen levels, and the decline of female hormone has been linked to elevated risk of CVDs in women for years; yet, some clinical trials concluded that estrogen treatment failed to improve the risk of CVDs in menopausal women (Hulley *et al.* 1998, Rossouw *et al.* 2002, Toh *et al.* 2010).

In contrast to estrogens, the effects of androgens on CVDs have rarely been studied, although early reports documented well that patients with CVDs have low serum testosterone (Dunajska *et al.* 2004, Turhan *et al.* 2007, Hu *et al.* 2011). As gender differences have been shown in CVDs with a higher incidence in males, androgens might promote CVDs. However, a protective role of androgens for CVDs was also reported (Malkin *et al.* 2003). A recent prospective study revealed that men whose total testosterone levels were in the lowest quartile were 40% more likely to die from CVDs than those with higher levels (Laughlin *et al.* 2008). Other studies showed that androgen deprivation therapy (ADT) led to increased risk of higher CVDs (Hu *et al.* 2012, Razzak 2012), and accordingly, the androgen replacement therapy (ART) has been applied and patients indeed showed improvement in heart functions (Vigna & Bergami 2005, Kang *et al.* 2012).

Due to this dilemma, recent studies were shifted to the effects of the androgen receptor (AR) rather than androgens on CVDs, as androgens might exert their effects through both AR and non-AR (non-genomic) actions (Heinlein & Chang 2002, Kang *et al.* 2004), as well as the fact that AR could be activated via both androgen- and non-androgen-mediated manners (Sugita *et al.* 2004). Importantly, the potential therapy of targeting AR in selective cells may have fewer side effects than targeting androgens using ADT.

Before the generation of *Ar*-knockout (ARKO) mice (Yeh *et al.* 2002), most androgen/AR studies in CVDs were performed with castrated mice or rats, which resulted in significant reduction of serum androgen levels (Malyusz *et al.* 1985, Alexandersen *et al.* 1999, Li *et al.* 2004, Henriques *et al.* 2008). However, the studies on castrated animals failed to directly address the AR effects on CVDs. Using the Cre-LoxP strategy, several mouse models with knockout of *Ar* in the whole body or in specific cell types, including general ARKO (GARKO, knockout in whole body), neuronal cell ARKO (NARKO) (Yu *et al.* 2013), macrophage lineage cell ARKO (MARKO), endothelial cell ARKO (EARKO), and smooth muscle cell ARKO (SARKO) were generated to study the impacts of cell-specific AR on CVDs (Yeh *et al.* 2002, Huang *et al.* 2014).

This review summarizes recent discoveries related to the effects of androgen/AR on CVDs, starting with hypertension then moving to the other CVDs, including stroke, myocardial hypertrophy, abdominal aortic aneurysm (AAA), and atherosclerosis, as well as metabolism.

Androgen/AR roles in hypertension

Hypertension or increased arterial blood pressure is one of the most prevalent CVDs affecting 25% of the adult population in the USA (Burt *et al.* 1995). Patients with long-term hypertension could also have increased risks of having other CVDs, including stroke, AAA, atherosclerosis, myocardial infarction, and congestive heart failure (Kannel 2000).

There are several risk factors linked to hypertension, such as age, obesity, alcohol abuse, and gender difference (Reckelhoff 2001). In human studies, men generally have higher blood pressure than women (Khoury *et al.* 1992, Burt *et al.* 1995, Wiinberg *et al.* 1995, Egan *et al.* 2010). However, the difference is gradually lost after women have passed menopause and men have declining androgen levels at ages around 70–79 years old (Burt *et al.* 1995). These observations suggest that sex hormones are involved in the progression of hypertension.

To clarify whether gender modulates hypertension, early studies have investigated the impact of gender on hypertension and demonstrated that male rats have higher blood pressure than females in several hypertensive rat studies (Iams *et al.* 1979, Ganten *et al.* 1989, Chen & Meng 1991), as well as the chemical-induced Dahl salt-sensitive (DS) rats (Rowland & Fregly 1992), deoxycorticosterone-salt-hypertensive rats (Ouchi *et al.* 1987), and the genetic mutation New Zealand hypertensive rats (Ashton & Balment 1991). In addition, several studies also showed similar observations in mouse models. Angiotensin II infusion significantly increased blood pressure in males, but to a lesser extent in females (Xue *et al.* 2005). The COX II-deficient mouse model also had higher blood pressures in males than females (Yang *et al.* 2005). From the human epidemiological and animal studies, it is clear that sex hormones modulate blood pressure.

Estrogens were originally proposed to have a protective role in blood pressure. Thus, several clinical trials have been proposed aiming to determine whether estrogens could inhibit blood pressure in postmenopausal women. However, the clinical studies failed to show convincing improvement after treating postmenopausal women with estrogen supplements (PEPI Trial Writing Group 1995, Rossouw *et al.* 2002, Anderson *et al.* 2004).

Interestingly, androgens gradually emerged as new candidates to account for the gender differences in CVDs. In older males, having decreased androgens and increased blood pressure, androgen supplement might reduce the blood pressure. However, the androgen treatment was found to exacerbate hypertension and increase the risk of

CVDs (Tangredi & Buxton 2001, Reckelhoff *et al.* 2005). In animal studies, Reckelhoff *et al.* made comparisons among male rats with various conditions, including non-castrated, castrated, anti-androgen flutamide-treated, and female rats. They found that the surgical and chemical castrations in these males reduced blood pressure levels down to the similar levels as observed in females (Reckelhoff *et al.* 1999). Similar results were reported in other castrated animal models (Malyusz & Ehrens 1983, Malyusz *et al.* 1985, Woods *et al.* 2010). In addition, Ely *et al.* (1991) found that the *Tfm* (*Ar*) rat, lacking functional AR, and castrated rats have lower blood pressure than intact control rats, suggesting that androgen/AR signaling might be involved in hypertension. Although there are still few epidemiological studies showing opposite results that testosterone is inversely correlated with blood pressure in male populations (Barrett-Connor & Khaw 1988, Khaw & Barrett-Connor 1988, Svartberg *et al.* 2004), it is generally accepted that males have higher blood pressure than females.

In summary, androgen/AR signaling would worsen hypertension and treatment with anti-androgens might be able to suppress hypertension. However, anti-androgen treatment has several side effects, including reduction of libido and suppression of sexual activities. These side effects may stop men who have hypertension from taking such treatments. Although anti-androgen or even the recently developed anti-AR compounds may inhibit high blood pressure, there are still controversial observations showing that ARKO mice developed higher blood pressure than wild-type mice (Huang *et al.* 2015) and that castration failed to prevent prenatally programmed hypertension. These complex outcomes suggest that the AR in individual cell types related to hypertension may have independent roles in the development of hypertension and using floxed *Ar* mice to delete AR in selective cells (Yu *et al.* 2008, Wang *et al.* 2009, Lin *et al.* 2011) may be a good tool to further study the role of AR in hypertension.

Androgen/AR roles in stroke

Stroke is a rapid loss in brain function due to ischemia caused by blockage or hemorrhage of blood vessels. There are three different kinds of strokes. Ischemic strokes happen when the blood vessels that supply the brain are occluded with a thrombus, a situation causing shortage or absence of delivery of oxygen and nutrition to the brain. Intra-cerebral hemorrhages, generally the least treatable, most disabling, and highest cause of

stroke death, occur when bleeding happens in the brain. Subarachnoid hemorrhages happen when blood spills into the subarachnoid spaces and is the least common type of stroke. Trauma and ruptured intracranial aneurysms account for 70–90% of subarachnoid strokes (Carwile *et al.* 2009).

Risk factors causing strokes include atherosclerosis, diabetes, gender, heart valve defects, and hypertension. Male stroke incidence rate is 33% and prevalence is 41% higher than female worldwide (Appelros *et al.* 2009). To account for this gender difference, the relationship between sex steroids and stroke has been discussed and analyzed in the cohort studies (Yeap *et al.* 2009, Morales 2010). Unexpectedly, the results showed that men with low testosterone levels had a higher incidence of stroke compared with those with normal testosterone levels. Another study also suggested that testosterone level is inversely associated with stroke severity and 6-month mortality (Jeppesen *et al.* 1996). In animal studies, it has been shown that testosterone helped with the functional recovery following stroke (Pan *et al.* 2005). Interestingly, treatment with high doses of testosterone worsened the stroke outcomes in the castrated mouse model and anti-androgen could reverse this exacerbated effect (Uchida *et al.* 2009). Other controversial outcomes have also been reported showing that testosterone increases lesion sizes in male rats with middle cerebral artery occlusions (Hawk *et al.* 1998, Yang *et al.* 2002). The treatment with the more potent androgen, dihydrotestosterone (DHT), exacerbated cerebral ischemia in male rats (Cheng *et al.* 2007). The effects of androgens on stroke are still controversial; thus, more comprehensive experiments will be needed in order to determine conclusive results of how androgens affect stroke.

In fact, controversial observations also occurred in patients with strokes. It has been reported that there is no difference regarding testosterone levels between post-stroke survival in young men and healthy men (Taggart *et al.* 1980). However, another study found that high endogenous testosterone increases the stroke incidence in children (Normann *et al.* 2009). In addition, prostate cancer patients treated with ADT also show the discrepancies in stroke. Gonadotropin-releasing hormone antagonist treatment increases stroke incidence; however, orchidectomy combined with androgen blockage and oral anti-androgen failed to show significant elevation in stroke incidence (Smith 2008, Keating *et al.* 2010, Azoulay *et al.* 2011, Collier *et al.* 2011).

From these controversial outcomes, it seems that androgens might affect stroke in differential ways

depending on several factors, such as age, methods of treatment with androgens or anti-androgens, and experimental approaches. However, these could not account for androgen/AR signaling effects on stroke, as androgen mainly exerts effects through AR. Even though androgens could affect cellular behavior through non-genomic actions (Wang *et al.* 2001, Heinlein & Chang 2002, Miyamoto *et al.* 2002, Walker 2003, Heinlein & Chang 2004, Kang *et al.* 2004, Foradori *et al.* 2008), the effects of stroke should show consistencies, but not paradoxes. One of the possible explanations for the differential effects of androgens on stroke may be that the AR plays differential roles in individual cells, thus affecting stroke in various manners. It would be an interesting future direction for the use of floxed AR mice (Yu *et al.* 2008, Wang *et al.* 2009, Lin *et al.* 2011) to clarify AR roles in different kinds of cells that are involved in cerebral injury. In summary, AR plays either protective or deleterious roles on strokes, dependent on several different kinds of factors.

Androgen/AR roles in atherosclerosis

Atherosclerosis progression can be a chronic expansion of the arterial intima with lipids, cells, and extracellular matrix for many years to decades long. Although this process itself, due to preservation of the arterial lumen, rarely leads to major symptoms, a few of these lesions undergo necrotic breakdown, which involves acute occlusive luminal thrombosis leading to myocardial infarction, unstable angina, sudden cardiac death, and/or stroke (Wolf *et al.* 2002).

Atherosclerosis is characterized as an inflammatory disease linked to certain risk factors, such as dyslipidemia, hypertension, and cigarette smoking. Atherosclerotic plaque progression and vulnerability are influenced by plaque cell and lipid composition rather than by the extent of arterial stenosis (Liu *et al.* 2003, Wu & von Eckardstein 2003, Kaufman & Vermeulen 2005). Vulnerable plaques in the coronary arteries tend to have a thin fibrous cap and a large lipid core with high macrophage content (Kyle *et al.* 2001). These plaques reside almost entirely in the intravascular space and, as a result of compensatory expansion of the diseased artery, are typically not detected by conventional angiographic approaches. Indeed, the majority of coronary occlusions and myocardial infarctions evolve from areas characterized by previous angiography as being mildly to moderately stenotic (Svartberg *et al.* 2006).

Gender differences also occur in atherosclerosis (Sinning *et al.* 2011). Misuse or abuse of androgens in athletes would increase the incidence of CVDs including atherosclerosis (Liu *et al.* 2003). However, the effects of exogenous testosterone on atherosclerosis are reported controversial in animal atherosclerotic models. Considering the fact that males have a higher incidence of atherosclerosis than females, it was originally believed that androgens promote atherosclerosis development. However, recent studies indicated that the physiological levels of androgens may inhibit atherosclerosis development. In an epidemiological study, atherosclerosis was shown to be inversely correlated with testosterone levels in men (Svartberg *et al.* 2006). Prostate cancer patients treated with ADT showed increased atherosclerosis (Shahani *et al.* 2008) and DHT administration could suppress atherosclerosis through inhibiting foam cell formation (Qiu *et al.* 2010). In contrast, controversial results were reported showing beneficial effects of castration in cholesterol-fed male rabbits (Larsen *et al.* 1993, Bruck *et al.* 1997, Alexandersen *et al.* 1999, Hanke *et al.* 2001). Although some studies suggest that androgen treatment might inhibit atherosclerosis, estrogen generated from high doses of testosterone may influence atherosclerosis development (Kushwaha & Hazzard 1981, Haarbo & Christiansen 1996). Therefore, it may not be easy to conclude how androgen and AR affect atherosclerosis progression based on these results.

What is the role of AR in atherosclerosis? No previous studies adequately addressed the role of AR on atherosclerosis. Recently, apolipoprotein E (*ApoE*)-null and GARKO mice have been developed for the investigation of the effects of ARKO on atherosclerosis (Ikeda *et al.* 2009), and the results suggest that GARKO mice have worse atherosclerosis progression than control mice. Interestingly, another study explored low density lipoprotein receptor (*Ldlr*)-null mice; with specific deletion of AR in selective cells, including monocytes/macrophages, endothelial cells, and smooth muscle cells, and found unexpected results (Huang *et al.* 2014). Knockout of AR in macrophages/monocytes suppressed atherosclerosis in *Ldlr*-null mice; however, knockout of AR in endothelial cells and smooth muscle cells did not show significant differences in atherosclerosis (Huang *et al.* 2014). Although cell-specific AR may differentially modulate atherosclerosis, GARKO mice still developed worse atherosclerosis than control mice in this study. These contrasting results could be due to the differential impacts of the serum androgen and lipid levels in these types of ARKO mice. GARKO mice have little serum testosterone; however, mice with knockout of

AR in selective cells have nearly normal androgen levels. As low androgen levels have been strongly linked to lipid production, this dramatic androgen difference may cause substantial changes in lipid profiles to alter atherosclerosis. Indeed, a study challenged *Tfm* mice with androgens and found reduced fatty streak formation. This result suggests that androgens might suppress lipid formation through non-genomic actions, which are independent of the AR (Nettleship *et al.* 2007).

Considering the fact that targeting androgen might lead to elevated lipid profiles, targeting AR in selective cells may represent a better therapeutic approach in atherosclerosis. Having shown promising results from cell-specific ARKO mice in suppressing atherosclerotic plaques, it would be of great interest to investigate compounds that could target AR in specific cell types. In fact, there has been one compound, ASC-J9, that has been previously demonstrated to be able to target AR in specific cell types without inhibiting androgen levels and fertility (Yang *et al.* 2007), including prostate cancer, liver cancer, bladder cancer, kidney cancer, wound healing, and SBMA neuronal disease (Miyamoto *et al.* 2007, Yang *et al.* 2007, Lai *et al.* 2009, Yamashita *et al.* 2012, Izumi *et al.* 2013, Lai *et al.* 2013, Liang *et al.* 2014). ASC-J9 could degrade AR via interrupting AR interaction with selective AR co-regulators including ARA55 and ARA70 (Lai *et al.* 2013). Naked AR, after being disassociated from the AR co-regulators, may then become more vulnerable to be degraded by the proteasome machinery that involves the alteration of E3 ubiquitin protein ligase-dependent degradation (Liang *et al.* 2014). Unlike the classic anti-androgens that have side effects on reducing libido and sexual ability, mice treated with ASC-J9 show normal sexual desire and fertility (Yang *et al.* 2007). Importantly, ASC-J9 treatment in mice with atherosclerosis results in some improvement of symptoms (Huang *et al.* 2014), suggesting the possibility of targeting AR in selective cells to restrict atherosclerosis progression.

In summary, the role of AR is distinctively different from androgens in atherosclerosis. As androgen deficiency would lead to elevated lipid profiles, targeting AR in selective cells without altering the androgen expressions may be a better strategy than targeting androgens in alleviating atherosclerosis.

Androgen/AR roles in abdominal aortic aneurysm (AAA)

An aneurysm has been defined as dilation of vessels and is a permanent dilation of 50% or more compared

with the normal diameter of the vessel (Grange *et al.* 1997, Johnston *et al.* 2002). An AAA is specifically located in the lower section of the aorta. There are several risk factors linked to AAA, including male gender, atherosclerosis, hypertension, and genetic predisposition (Annambhotla *et al.* 2008, Weintraub 2009). Up-to-date, surgery is the only treatment and there is no effective medicine to treat AAA in patients (Greenhalgh & Powell 2008). Early studies suggested that some molecular mediators and extracellular matrix-degrading proteinases, including $\text{INF}\gamma$, CXCL10, CCR2, JNK, $\text{TGF}\beta$ 1, ERK, the matrix metalloproteinase (MMP) family, and angiotensins (Thompson & Baxter 1999, Daugherty *et al.* 2001, Yoshimura *et al.* 2005, MacTaggart *et al.* 2007, King *et al.* 2009, Zhang *et al.* 2009, Habashi *et al.* 2011, Holm *et al.* 2011), might contribute to AAA progression. However, recent studies demonstrated that MMPs (especially MMP-2 and MMP-9) are highly expressed in human and experimental mouse models of AAA (Sakalihan *et al.* 1996, Goodall *et al.* 2001, Longo *et al.* 2005, Pearce & Shively 2006). Although MMPs might be the potential reason behind AAA development, the molecular mechanisms related to gender differences in AAA remain unclear.

Male gender is one of the risk factors involved in AAA initiation and progression. Males show higher mortality rate, rupture risk, and vessel dilation than females after surgical therapy (Katz *et al.* 1997, Forbes *et al.* 2006, Hannawa *et al.* 2009). Henriques *et al.* (2004) used angiotensin II-induced AAA mouse model to study the effects of sexual hormones on AAA initiation and progression and found that castration in male mice reduced AAA incidence from 85 to 18%; however, ovariectomy did not alter AAA incidence in female mice. Similarly, another study shows that castration reduced AAA diameters, testosterone treatment restored aortic diameters to the extent before castration (Cho *et al.* 2009), and that ovariectomy did not alter the aortic diameters in female rats with AAA. Those studies suggested that androgen might play a dominant role in AAA initiation and progression, and this speculation is also supported by epidemiological studies, as males show higher incidence, mortality, and aortic diameter in AAA than females. Similarly, other studies used exogenous androgens to treat AAA mice and rats and found that this androgen treatment increases the AAA incidence in male and female mice as well as male rats (Henriques *et al.* 2008, Cho *et al.* 2009). Together, androgen/AR signaling stimulates AAA initiation and progression.

To further separate androgen and AR effects on AAA development, Bourghardt *et al.* implanted testosterone pellets in GARKO-*ApoE*-null mice, and found that depletion of AR has protection effects on AAA development and androgen treatment could promote AAA development in WT mice, but not in GARKO mice (Bourghardt 2010). Another group also developed several cell-specific ARKO mice that had AR knocked out in myeloid lineage, smooth muscle cells, or endothelial cells with *ApoE*-null background (Kobayashi *et al.* 2003, Alva *et al.* 2006, Yu *et al.* 2011). These cell-specific ARKO mice were treated with angiotensin II to induce AAA formation, and the obtained results show that the GARKO mice did not develop AAA (Huang *et al.* 2015). Similar results were also observed in MARKO and SARKO mice, but not in EARKO mice (Huang *et al.* 2015), suggesting that AR might modulate AAA development through altering inflammation and integrity of the aortic wall. Nevertheless, results from these ARKO mouse studies concluded that AR signaling is essential in AAA development.

Mechanistically, AR modulates AAA formation through inflammation via elevating interleukin 1 alpha (IL-1 α) and fibrosis process via mediating transforming growth factor beta (TGF β 1). Although TGF β 1 has been recently proposed as a therapeutic approach in treating AAA progression, TGF β 1 has also been indicated as a deleterious factor in AAA development (King *et al.* 2009). Future studies would be needed in order to clarify the actual role of TGF β 1 in AAA development.

Actually, MARKO and SARKO mice do not have altered androgen levels and still show inhibition in AAA development and progression. In addition, GARKO mice showed complete abolishment in AAA incidence; however, castration in male mice showed around 20% incidence in AAA initiation. These intriguing results suggest that AR is indispensable in AAA development. It is interesting to see whether targeting AR with ASC-J9, the AR degradation enhancer, or other small molecular AR inhibitors has the therapeutic potential in treating AAA.

Androgen/AR role in cardiac hypertrophy

Cardiac hypertrophy is prevalent in a substantial portion of individuals with hypertension (Devereux *et al.* 1987, Kaplinsky 1994) and recognized as an independent risk factor for congestive heart failure and sudden cardiac death (Neyses & Pelzer 1995). Extended cardiac fibrosis results in increased myocardial stiffness, causing ventricular

dysfunction, and, ultimately, heart failure (Weber & Brilla 1991).

Significant gender-related differences in CVDs were already described in earlier sections. Men are at greater risk for cardiac hypertrophy than age-matched women (Fiebach *et al.* 1990, Crabbe *et al.* 2003, Maron *et al.* 2003). It was reported that the male heart of many species is hypertrophied relative to the female heart. In addition, the difference in repolarization on the electrocardiogram (ECG) between men and women has suggested that women have a QT (QT interval is a measure of the time between the start of the Q wave and the end of the T wave) prolongation relative to men (Lepeschkin & Surawicz 1953). The difference in QT duration is not evident before puberty; however, it was persistent after menopause (Alimurung *et al.* 1950, Rautaharju *et al.* 1992, Stramba-Badiale *et al.* 1995). Other factors can also modify disease outcome. Age is the most commonly reported factor that is associated with the extent and severity of left ventricular hypertrophy (Klues *et al.* 1995, Maron *et al.* 2003). Younger patients tend to have significantly more hypertrophy than older patients, and hypertrophy in older patients is generally more localized (Klues *et al.* 1995).

Despite the evident gender differences in cardiac phenotypes, which are probably dependent on sex hormones, little is known about the underlying mechanisms. Up-to-date, there has been no clear evidence that androgens can produce direct hypertrophic effects on cardiac myocytes independently from other neurohormonal or hemodynamic effects (Sadoshima & Izumo 1993, Marsh *et al.* 1998). Nonetheless, the differences in hypertrophic response with sex hormone treatments have been observed. Treatment with estrogens were shown to play a protective role in the hypertrophic response (Xin *et al.* 2002), whereas exposure of cardiac myocytes to androgens results in hypertrophy (Marsh *et al.* 1998). The results of both *in vitro* and *in vivo* studies indicate that sex hormones play a key role in the development of cardiac structural abnormalities. Estrogens showed anti-proliferative effects on cardiac fibroblasts (Dubey *et al.* 1998) and vascular smooth muscle cells (Chen *et al.* 1996, Somjen *et al.* 1998), whereas androgens increase proliferation of vascular smooth muscle cells (Fujimoto *et al.* 1994). Studies using sinoaortic denervation-induced cardiac hypertrophy in rats have also shown that testosterone facilitated hypertrophy while estrogen inhibited it (Cabral *et al.* 1988). However, a less severe model of cardiac hypertrophy in rats (swimming- or hypertension-induced) failed to confirm the

anti-proliferative effect of estrogen (Malhotra *et al.* 1990). Moreover, not all males developed gender-related cardiac abnormalities. Somjen *et al.* (1998) reported a biphasic proliferative response for both estrogen and testosterone in vascular smooth muscle and endothelial cells.

Mice lacking guanylyl cyclase A (GC-A), a natriuretic peptide receptor, exhibit salt-resistant hypertension, myocardial hypertrophy, interstitial fibrosis, and sudden death (before the age of 6 months) (Lopez *et al.* 1995). The male GC-A (*Npr1*) KO mice show more pronounced cardiac hypertrophy and fibrosis compared with the female GC-A KO mice, and these gender-related differences were not observed in wild-type (WT) mice. Additionally, these gender-related differences were attenuated either by castration or the treatment with the anti-androgen, flutamide, and were abolished by a genetic disruption of angiotensin (Ang) II type 1A (AT1A) receptors in the male GC-A KO mice.

AR is present in the myocardial tissues (Marsh *et al.* 1998, Meyer *et al.* 1998, Weinberg *et al.* 1999), which allows androgens to modulate the cardiac phenotype and produce hypertrophy by direct receptor-specific mechanisms that are involved in the modulation of many genes (Morano *et al.* 1990, Towbin & Lipshultz 1999). Marsh *et al.* (1998) investigated whether AR in myocytes could regulate cardiac hypertrophy by exogenous androgens treatments and found a significant hypertrophic response directly in cardiac myocytes. When they examined the hypertrophic response by using [³H] phenylalanine incorporation and atrial natriuretic peptide secretion as markers of hypertrophy in cultured rat myocytes, they found that both testosterone and DHT produced an AR-specific hypertrophic response in these cells. Importantly, DHT is the most active natural androgen, which is never aromatized to estrogen. So, it is evident that AR can modulate hypertrophic response in cardiac myocytes.

All together, men have increased cardiac hypertrophy compared with age-matched women and androgens would promote cardiac hypertrophy.

Androgen/AR roles in heart failure

Myocardial hypertrophy with hypertension represents a risk factor for congestive heart failure. Heart failure can also result from CADs and ischemia due to the potential mechanisms involved in maladaptive and prolonged neurohormonal and pro-inflammatory cytokine activation that result in a metabolic shift favoring catabolism and

loss of skeletal muscle bulk and function (Malkin *et al.* 2006b).

Men have a higher incidence and severity of heart failures than women (Fairweather *et al.* 2008, Wexler *et al.* 2009, Regitz-Zagrosek *et al.* 2010). The incidence of myocarditis is higher in men than in women, and notably, gender difference in myocardial ischemia/reperfusion injury has also been established (Wang *et al.* 2005a, 2008). Indeed, some studies have clearly indicated that testosterone exacerbates ischemia/reperfusion-induced cardiac dysfunction and enhances myocardial inflammation and apoptotic signaling in male hearts (Cavasin *et al.* 2003, Wang *et al.* 2005b).

However, Pastor-Perez *et al.* (2011) investigated sex hormonal levels in men with chronic heart failure and found that 28% were deficient of testosterone, and less circulating testosterone levels in the body have been related to exercise capability in male patients with chronic heart failure (Manzano-Fernandez *et al.* 2011).

Recently, several reports of follow-up studies in prostate cancer patients who received ADT revealed that these patients have a higher risk of developing heart failure (Keating *et al.* 2006, Shahani *et al.* 2008, Keating *et al.* 2010, Chung *et al.* 2011, Martin-Merino *et al.* 2011, Nguyen *et al.* 2011, Collier *et al.* 2012) and cardiovascular mortality (Tsai *et al.* 2007), suggesting that loss of androgen might promote heart failure. In contrast, ART has been proposed to improve heart function (Malkin *et al.* 2006a,b), with the results showing a significant increase in cardiac output, and an improved functional capacity and symptoms of men with heart failure (Pugh *et al.* 2003, 2004, Malkin *et al.* 2006b). Other reports also suggested that androgen levels might also have an anti-ischemic effect on heart function improvement in clinics (Rosano *et al.* 1999, Webb *et al.* 1999a,b).

The underlying mechanisms of the androgen effects on heart failure remain unclear; however, a variety of processes related to cardiac function have been proposed. For example, androgen might be able to influence the contractile function of myocardium, endothelial function, and alterations in skeletal muscle (Vicencio *et al.* 2006, Malkin *et al.* 2009, Rowell *et al.* 2009). Androgen is also involved in nervous system development (Bialek *et al.* 2004), which could increase neuritic growth and synaptogenesis in both motor neurons of the spinal nucleus of the bulbocavernosus (Forger *et al.* 1992, Matsumoto 1997) and some pelvic autonomic neurons (Meusburger & Keast 2001), as well as increase nerve growth factor levels in the brain (Tirassa *et al.* 1997).

It seems that both extreme high or low androgen levels increase the chance of heart failure. As it is well known that testosterone abuse in athletes significantly raises the chance of sudden heart failure and the recent epidemiology studies show that castrated prostate cancer patients have elevated risk of developing CVDs, suggesting the androgen levels might need to be balanced. Therefore, androgen/AR signaling might not be the suitable therapeutic target in heart failure patients.

AR effects on the metabolic syndrome/diabetes and their impacts on CVDs

Earlier we discussed that several factors, such as smoking, hypertension, diabetes, and hyperlipidemia, can affect the occurrence of CVDs. These factors are also known to affect the metabolic syndromes (Julius *et al.* 1981, Schulze *et al.* 1981). It is well known that obese individuals are prone to develop more insulin resistance compared with non-obese individuals, as these individuals have higher chances of having metabolic risk factors, such as elevated circulating triglycerides (TGs), reduced high-density lipoprotein cholesterol levels, elevated fasting blood glucose levels, and high blood pressure (Hu *et al.*

2004). These metabolic abnormalities in conjunction with abdominal obesity represent the classical symptoms of the metabolic syndrome. Therefore, CVDs are considered to be closely correlated with the metabolic syndrome.

Lin *et al.* investigated whether the androgen/AR affects the metabolic syndrome, by determining whether GARKO mice have variations in their lipid metabolism and insulin/leptin resistance. It was shown that the male GARKO mice have increased body weight gain compared with their WT littermate control mice (Lin *et al.* 2005). The observed obesity in GARKO male mice was shown to be associated with elevations of circulating TGs and free fatty acids, as well as lipid deposition in non-adipose tissue, including the liver and muscle (Lin *et al.* 2005). Consistent with the enlarged fat mass, circulating leptin was shown elevated in the GARKO male mice and was observed before the onset of obesity (Lin *et al.* 2005). These results suggest that global loss of AR increased circulating leptin levels independent of body fat accumulation.

Lin *et al.* further treated the GARKO mice and their WT littermates with DHT for analysis of several serum hormones and metabolic parameters. Surprisingly, DHT replacement could not reverse the metabolic abnormalities and insulin resistance in GARKO male mice (Lin *et al.* 2005), suggesting that the AR is critical in

Table 1 Summary of gender differences and androgen and anti-androgen treatments in cardiovascular diseases.

CVDs	Gender difference	Androgen or anti-androgen treatments in clinical outcomes	References
Hypertension	Males have higher blood pressure than females	Androgen treatments increase blood pressure in male patients	Devereux <i>et al.</i> (1987), Daugherty <i>et al.</i> (2001), Dunajska <i>et al.</i> (2004), Collier <i>et al.</i> (2012)
Stroke	Males have higher incidence in stroke than females	Gonadotropin-releasing hormone agonist treatment increases stroke incidence in prostate cancer patients, but orchiectomy combined with androgen blockage and oral anti-androgen failed to show significant elevation in stroke incidence	Julius <i>et al.</i> (1981), Kannel (2000), Wang <i>et al.</i> (2005), Kang <i>et al.</i> (2012), Zimmerman (2012)
Atherosclerosis	Males have thicker intima-media in early carotid atherosclerosis than females	Androgen deprivation therapy in patients with prostate cancer resulted in increased atherosclerosis	Alimurung <i>et al.</i> (1950), Kyle <i>et al.</i> (2001), King <i>et al.</i> (2009), Lai <i>et al.</i> (2009)
Abdominal aortic aneurysm	Males have higher incidence than females	N/A	Neyses & Pelzer (1995), Nettleship <i>et al.</i> (2007), Nguyen <i>et al.</i> (2011)
Myocardial hypertrophy	Males are at greater risk for myocardial hypertrophy than age-matched females	N/A	Reckelhoff (2001), Reckelhoff <i>et al.</i> (2005), Lai <i>et al.</i> (2009)
Myocardial infarction	Females develop more severe myocardial infarction than males	Gonadotropin-releasing hormone agonist treatment increases the risk of incident myocardial infarction and sudden cardiac death	Walker (2003), Vigna & Bergami (2005), Wang <i>et al.</i> (2005a), Lai <i>et al.</i> (2009), Razzak (2012)

N/A, data not available.

Myocardial infarction (MI)GARKO
MI**Stroke**GARKO
Stroke**Hypertension**Androgen ↑
Hypertension**Atherosclerosis**GARKO
Artherosclerosis ↑**Abdominal aortic aneurysms (AAA)**GARKO
AAA ↓**Macrophages**MARKO
Artherosclerosis ↓**Macrophages**MARKO
AAA ↓**Vascular smooth muscle cells**SARKO
Artherosclerosis —**Vascular smooth muscle cells**SARKO
AAA ↓**Vascular endothelial cells**EARKO
Artherosclerosis —**Vascular endothelial cells**EARKO
Aneurysms —

↑ Promotion ↓ Inhibition — No effect

GARKO, General androgen receptor knockout; MARKO, macrophage specific androgen receptor knockout; SARKO, smooth muscle cell specific androgen receptor knockout; EARKO, endothelial cell specific androgen receptor knockout

Figure 1

The impact of knocking out cell-specific AR on the development and progression of CVDs. A full colour version of this figure is available at <http://dx.doi.org/10.1530/JOE-15-0518>.

mediating the effects of androgens to regulate glucose and lipid metabolisms in males.

From these results, it was concluded that visceral obesity and progressive insulin resistance are two major abnormalities linked to the metabolic syndrome. Lin *et al.* (Hoesche *et al.* 1993, Lin *et al.* 2008) further developed tissue-specific ARKO mouse models, including liver-specific

(LARKO) and NARKO mice, as these organs are considered to be critical in these two processes. They were then able to evaluate the effect of AR in these specific tissues on the metabolic syndrome. The LARKO mice were developed using albumin-Cre mice and fed a high-fat diet (HFD) and mice were shown to weigh 13% more than their WT littermates (Lin *et al.* 2008), suggesting that the LARKO mice

were more susceptible to diet-induced obesity. The LARKO mice fed HFD exhibited impaired glucose metabolism due to the developed insulin resistance (Lin *et al.* 2008).

Interestingly, several groups demonstrated that there are differential insulin sensitivities in the male and female central nervous system (Obici & Rossetti 2003, Schwartz & Porte 2005, Clegg *et al.* 2006). The NARKO mice were developed using synapsin I-Cre mice (Hoesche *et al.* 1993), and the NARKO male mice displayed increased body weight with increased visceral adiposity, hyperinsulinemia, hyperglycemia, and increased hepatic glucose production. In addition, the NARKO mice exhibited impaired insulin responsiveness in the hypothalamus (Yu *et al.* 2013).

From these studies of global ARKO mice and tissue-specific ARKO mice, it can be concluded that AR plays an important role in contributing to the obesity and insulin/leptin resistance and can therefore increase the metabolic syndromes in mice.

Summary

CVDs are related to each other and have common features. They all show gender differences (Table 1); however, the results of the androgen therapies, either ADT or ART, applied in all CVDs are not conclusive. Even whether the roles of androgens are protective or deleterious are not conclusive. In addition to this inconsistency, modulating body androgen levels is expected to cause many other side effects. So, it seems essential to develop another strategy of targeting androgen/AR for better treating the CVDs. It may be the time to turn our focus to target the AR rather than androgens. The therapy of targeting AR in selective cells might have several advantages over androgen therapy: (1) it will not affect whole-body androgen levels, thereby can avoid unwanted side effects; (2) it may not affect body lipid profiles, which can affect some CVDs; (3) we may target AR in selective cell types, not the whole body; and (4) we may also target the non-androgen-mediated AR action.

The results of targeting AR in atherosclerosis and AAA using cell-specific ARKO mouse models indicate that targeting AR is indeed a better approach to battle these diseases (Fig. 1). Obvious improvement in AAA in the GARKO mice was observed. Interestingly, it was found that the ARKO effect on the total body was opposite to the monocyte/macrophages-specific ARKO effect on atherosclerosis. This result implies that AR contribution in each cell type in each disease might be different.

Taken together, targeting AR, instead of androgens, emerges as a new therapeutic approach in CVDs. However, the most critical point is how to apply this concept into clinical approach to target AR in specific cell types in CVDs. The use of the specific AR degradation enhancer, ASC-J9, or other newly developed small molecules that target AR may make it possible to target AR in specific cell types without affecting body androgen levels, thus reducing the development of the unwanted side effects.

Declaration of interest

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

Disclosure

ASC-J9[®] was patented by the University of Rochester, the University of North Carolina, and AndroScience, and then licensed to AndroScience. Both the University of Rochester and C.C. own royalties and equity in AndroScience.

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