



Review

Androgens and Androgen Receptors as Determinants of Vascular Sex Differences Across the Lifespan

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ABSTRACT

Androgens, including testosterone and its more potent metabolite dihydrotestosterone, exert multiple actions in the body. Physiologically, they play a critical role in male sex development. In addition, they influence vascular function, including arterial vasodilation and mediation of myogenic tone. Androgens are produced from 9 weeks' gestation in the human fetal testis, as well as in small amounts by the adrenal glands. Serum concentrations vary according to age and sex. The vasculature is a target for direct actions of androgens, which bind to various sex hormone receptors expressed in endothelial and vascular smooth muscle cells. Androgens exert both vasoprotective and vaso-injurious effects, depending on multiple factors including sex-specific effects of androgens, heterogeneity of the vascular endothelium, differential expression of androgen and sex hormone receptors in endothelial and vascular smooth muscle cells, and the chronicity of androgen administration. Long-term administration of androgens induces vasoconstriction and influences endothelial permeability, whereas acute administration may have opposite effects. At the cellular level, androgens stimulate endothelial cell production of nitric oxide and inhibit proinflammatory signalling pathways, inducing vas-

RÉSUMÉ

Les hormones androgènes, y compris la testostérone et son métabolite plus puissant, la dihydrotestostérone, exercent de multiples actions dans l'organisme. D'un point de vue physiologique, elles jouent un rôle déterminant dans le développement sexuel des hommes. De plus, elles influencent la fonction vasculaire, notamment la vasodilatation artérielle et la médiation du tonus myogène. Les androgènes sont produits à partir de la 9^e semaine de gestation dans les testicules du fœtus humain, ainsi qu'en petites quantités par les glandes surrénales. Les concentrations sériques varient en fonction de l'âge et du sexe. Le système vasculaire est la cible d'actions directes des androgènes, qui se lient à divers récepteurs d'hormones sexuelles exprimés à la surface des cellules des muscles lisses vasculaires et endothéliaux. Les androgènes exercent à la fois un effet protecteur et délétère sur le système vasculaire, en fonction de nombreux facteurs, dont le sexe, l'hétérogénéité de l'endothélium vasculaire, l'expression différentielle des androgènes et des récepteurs d'hormones sexuelles sur les cellules des muscles lisses vasculaires et endothéliaux, et la chronicité de l'administration d'androgènes. L'administration prolongée d'androgènes provoque la vasoconstriction et influence la perméabilité

Androgens are steroid hormones that are critically involved in normal male sex development during the fetal period as well as in the development of secondary sexual characteristics during puberty. Growing evidence indicates that they also have significant roles within the vasculature, including arterial vasodilation, mediation of myogenic tone, and inhibition of neointimal atherosclerotic plaque formation.¹

Despite increasing awareness and ongoing public health measures, cardiovascular disease (CVD) remains the leading cause of death worldwide, representing more than 30% of all deaths and 45% of noncommunicable deaths.² Hypertension, in particular, occurs and progresses differently in men and women, as reviewed in Gillis et al.³ There are several strands of evidence that demonstrate that the sex hormones, androgens, and estrogens account for some of the sexual dimorphism related to hypertension and CVD. Although estrogens also have significant effects on the vasculature, this review will focus purely on androgen production across the lifespan and the role of androgens and androgen receptors in vascular function from the fetal period to adulthood.

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Androgen Production Throughout the Lifespan

Testosterone is the principal male androgen and is synthesized from cholesterol primarily by the Leydig cells of the testes in males to a varying extent across the lifespan, as shown

orelaxation and vasoprotection. However, androgens also activate endothelial production of vasoconstrictors and stimulate recruitment of endothelial progenitor cells. In humans, both androgen deficiency and androgen excess are associated with increased cardiovascular morbidity and mortality. This review discusses how androgens modulate vascular sex differences across the lifespan by considering the actions and production of androgens in both sexes and describes how cardiovascular risk is altered as levels of androgens change with aging.

in Figure 1. Small amounts are also secreted by the zona reticularis of the adrenal glands in both sexes.⁴ Production of testosterone by the human fetal testis begins at approximately 9 weeks, with a peak between 14 and 17 weeks, and then a sharp decline, so that in late pregnancy the serum concentration of testosterone is similar in male and female fetuses.⁵ In both sexes, there is also a prenatally active alternative androgen biosynthesis pathway before 17 α -hydroxyprogesterone to 5 α -dihydrotestosterone, which bypasses testosterone with increased activity in congenital adrenal hyperplasia variants, resulting in virilization in female babies.⁶ From 6 to 10 days after birth, gonadotrophin-releasing hormone (GnRH) starts to rise, resulting in minipuberty. During this time, luteinizing hormone (LH) reaches pubertal levels by postnatal days 16 to 20.⁷ In boys, serum testosterone levels peak between 1 and 3 months of age and then decline by approximately 50% per month to prepubertal levels by 9 to 12 months of age.⁸ Although girls primarily have a peak of estrogen production during minipuberty, serum testosterone levels also increase with some overlap between serum concentrations in both sexes.⁸

After minipuberty, there is a childhood pause of steroid production, with small irregular GnRH pulses and low gonadotrophins⁹ until onset of puberty. Puberty typically commences between the ages of 9 and 14 years in boys, with a rapid rise in serum testosterone levels, which are then maintained into adulthood. Beyond age 30 years, testosterone levels decrease at a rate of 1% to 2% per year, with some variation according to other lifestyle factors, such as adiposity, medications, and chronic disease.¹⁰ Epidemiologic studies have shown that approximately 40% of men above the age of 45 years and 50% of men above 80 have low circulating testosterone levels.¹¹

In girls, menarche is usually reached by age 13 years, with the first signs of puberty being noted from age 8 years, although there is a secular trend to these milestones occurring earlier.¹² In girls, serum testosterone levels increase during puberty to a peak between the ages of 20 and 25 years and then gradually decline with age, although at all ages, testosterone remains < 2 nmol/L.¹³ In adulthood, the physiological levels of testosterone in men range from 10 to 30 nmol/L with lower levels found in women (0.6 to 2.5 nmol/L).¹⁴

endothéliale, tandis qu'une administration à court terme pourrait avoir les effets opposés. À l'échelle cellulaire, les androgènes stimulent la production d'oxyde nitrique par les cellules endothéliales et inhibent les voies de signalisation pro-inflammatoires, ce qui entraîne une vasorelaxation et une vasoprotection. Toutefois, les androgènes activent également la production endothéliale de vasoconstricteurs et stimulent le recrutement des cellules progénitrices endothéliales. Chez l'humain, le déficit tout comme l'excès d'androgènes sont associés à une augmentation de la morbidité et de la mortalité cardiovasculaires. Nous nous penchons ici sur la façon dont les androgènes modulent les différences vasculaires liées au sexe tout au long de la vie, en prenant en considération les actions et la production d'androgènes chez l'homme et chez la femme, et nous décrivons comment le risque cardiovasculaire change à mesure que les taux d'androgènes varient avec le vieillissement.

The Androgen Receptor

Approximately 7% of testosterone is converted to the more potent androgen, dihydrotestosterone (DHT) via the enzyme 5- α reductase. In adult men, testosterone production ranges from 3 to 7 mg per day¹⁵ and DHT production from 0.2 to 0.3 mg per day.¹⁶ DHT is more biologically active than testosterone, with a 2-fold higher affinity for the androgen receptor (AR) and a 5-fold reduction in dissociation rate compared with testosterone.¹⁷ In addition, the enzyme aromatase metabolizes approximately 0.5% of testosterone to estrogen, accounting for 95% of circulating estrogen in men.¹⁸ To regulate target gene transcription, testosterone and DHT can bind to the AR in a DNA binding-dependent (genomic or classical) manner, resulting in new protein synthesis or in a non-DNA binding-dependent (nongenomic or nonclassical) manner, leading to a rapid induction of secondary messengers to initiate cellular events, such as protein phosphorylation^{19,20} (Fig. 2).

The AR is a single copy gene located on the X chromosome at Xq11-12. Its structure is similar to that of other nuclear receptors and consists of 3 functional domains: the N-terminal domain (NTD); the DNA binding domain (DBD); and the C-terminal ligand binding domain (LBD). The NTD is the most variable domain and is responsible for transcription regulation. It is encoded by exon 1 and contains a polyglutamine (CAG) sequence. This has been demonstrated to be of functional importance because AR activity is inversely proportional to the number of CAG repeats, with the normal sequence described as 11 to 31 triplets.²¹ Exons 2 and 3 of the AR encode the DBD, which is the most highly conserved region.²² The DBDs of all sex steroid receptors consist of 2 zinc fingers that facilitate direct DNA binding of the AR to the promoter and enhancer regions of AR-regulated genes, allowing activation of the NTD and LBD for gene transcription.²² Meanwhile, exons 4 to 8 encode the LBD,²³ and this also has a similar structure to other sex steroid receptors. The LBD regulates the interactions between the AR and the heat shock and chaperone proteins and also interacts with the NTD to stabilize bound androgens.²² More than 400 mutations and polymorphisms in the AR have been described,²⁴ and these are thought to be inversely proportional to the transcriptional response to testosterone.²⁵

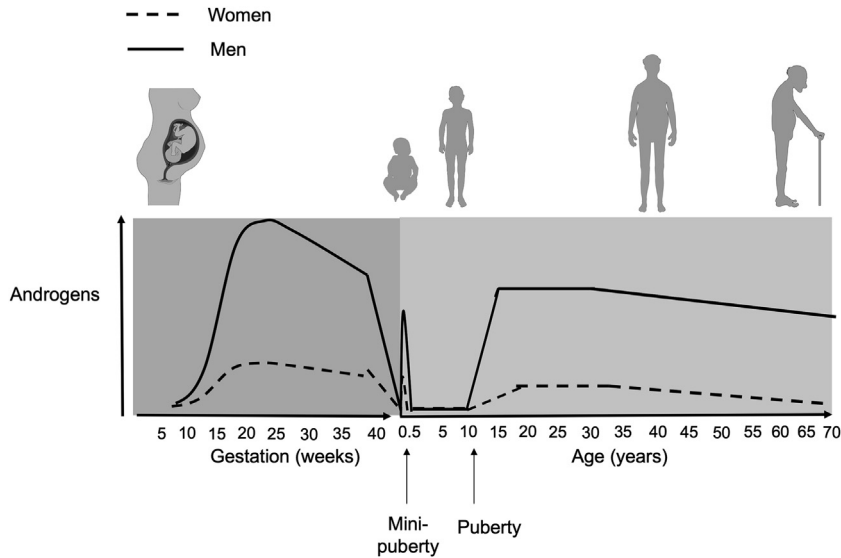


Figure 1. Representative of androgen levels across the lifespan in men and women.

Androgen Signalling Through the AR

Without ligand binding, the AR is primarily found in its basal state in the cytoplasm. Here, it associates with heat shock proteins (HSPs), which modulate AR conformation in preparation for efficient ligand binding.²⁶ Androgens cross the plasma membrane, enter the cytoplasm, and bind to the AR. This leads to the dissociation of chaperone proteins and translocation of the complex to the nucleus, where it dimerizes

and binds to androgen-response elements (AREs) to modulate gene transcription. AR binding to specific AREs results in recruitment of histone acetyltransferase (HAT) enzymes and other essential coregulators.²⁶ This facilitates binding of TATA binding protein (TBP), followed by general transcription factors to begin transcription and to regulate the expression of androgen-regulated genes.^{26,27} Protein synthesis subsequently occurs.¹⁷

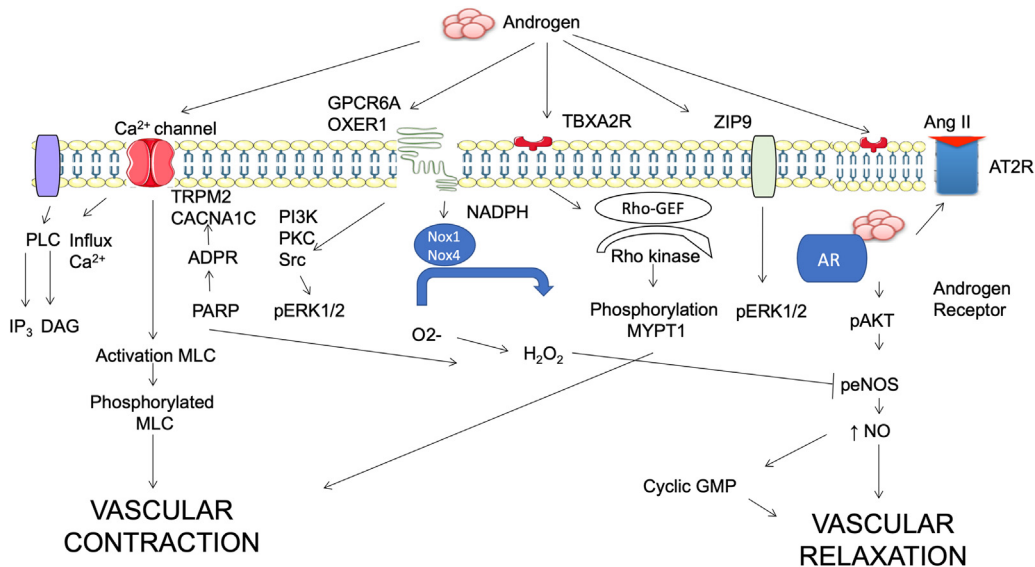


Figure 2. Genomic and nongenomic actions of androgens in the vascular smooth muscle cell (adapted from reference 20). ADPR, ADP-ribosyl; Ang II, angiotensin II; AR, androgen receptor; AT2R, angiotensin II type-2 receptor; CACNA1C, calcium voltage-gated channel subunit alpha1 C; CPLC, phospholipase C; DAG, diacylglycerol; eNOS, endothelial NO synthase; GPCR, G-protein coupled receptor; H₂O₂, hydrogen peroxide; IP₃, inositol trisphosphate; MLC, myosin light chain; MLCK, myosin light chain kinase; MYPT1, myosin phosphatase target subunit 1; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; O₂⁻, superoxide anion; pAKT, phosphorylated protein kinase B; PARP, poly ADP-ribose polymer; peNOS, phosphorylated endothelial NO synthase; pERK 1/2, phosphorylated extracellular signal-regulated kinases 1/2; PI3K, phosphoinositide 3-kinase; PKC, protein kinase; Src, proto-oncogene tyrosine-protein kinase; TBXAR, thromboxane A2 receptor; TRPM2, transient receptor potential cation channel subfamily M member 2.

Actions of Androgens via Androgen-Responsive Membrane Receptors

G-protein-coupled receptor family C group 6 member A (GPC6A) is a member of the Family C, G-protein coupled receptors. It is expressed in many tissues, including bone marrow stromal cells, monocytes, prostate cancer cells, skeletal muscle cells, vascular smooth muscle cells, endothelial cells and Leydig cells.²⁸ It comprises a venus fly trap (VFT) motif attached to a 7-transmembrane domain and is activated by multiple ligands including calcium, zinc, magnesium, l-arginine, l-lysine, l-ornithine, and the bone-derived peptide osteocalcin.²⁹

In vivo, testosterone-induced ERK phosphorylation in the bone marrow and testes is markedly attenuated in GPC6A $-/-$ mice, demonstrating that GPC6A is a nonclassical receptor through which androgens induce ERK activation.³⁰ ERK activity is rapidly stimulated by androgens in HEK-293 cells (which lack both the AR and GPC6A receptor) transfected with GPC6A. This rapid activation occurs in a calcium-dependent manner and does not take place in nontransfected HEK-293 controls.³⁰ Flutamide, an AR inhibitor, has no effect on testosterone-stimulated GPC6A activation of phospho-ERK. Androgens do not stimulate ARE-luciferase activity in HEK-293 cells expressing only GPC6A but do stimulate HEK-293 cells transfected with AR, suggesting activation of nuclear receptor signalling.

In addition to GPC6A, non-DNA binding-dependent actions of testosterone are associated with androgen binding to ZIP9, a membrane-integrated receptor.³¹ ZIP9 acts as both a membrane AR and zinc transporter. In 93RS2 Sertoli cells, a cell line that does not express AR, testosterone at 10 nM induces ERK1/2 phosphorylation. Similar effects were also observed in CREB and ATF-1 phosphorylation, effects suppressed by ZIP9-siRNA, indicating that ZIP9 is involved in the testosterone-induced signalling pathway. Apoptosis is altered by ZIP9, with a reduction seen in cells transfected with ZIP9.³²

A third membrane receptor, which can be activated by androgens is G protein coupled oxo-eicosanoid receptor 1 OXER1 (5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid receptor). This is a GPCR, first described in 2002, that regulates the biological actions of 5-oxo-eicosatetraenoic acid (5-oxoETE), a product of the metabolism of arachidonic acid by 5-lipoxygenase (5-LOX) and peroxidase.³³ It is expressed in liver, kidney, spleen, lung, and various inflammatory cells including eosinophils, neutrophils, lymphocytes, and monocytes.³³ Testosterone acts as an antagonist of this receptor, opposing the p38 and PI3K pathways, thereby modulating cell migration and metastasis in DU-145 prostate cancer cells.³⁴

Nongenomic Actions of Androgens

Nongenomic signalling is independent of the ligand-dependent transactivation function of nuclear receptors and typically occurs within a short time frame.³⁵ To be considered a nongenomic response, the androgen-induced response must occur in a time frame not long enough to allow gene transcription: normally, within seconds to minutes. The response should be observed even when the androgen is conjugated to

molecules such as bovine serum albumin (BSA) that prevent it from entering into the cytoplasm. In addition, the nongenomic response should not be blunted by inhibitors of transcription and does not require a functional nucleus or transcription-translation machinery activation.¹⁹ The nature of the nongenomic actions of androgens depend on the type of target cell but can include rapid Ca^{2+} release, activation of the RAS/MEK/ERK MAPK pathway, or modification of ion channels.¹⁹

The rapid rise of intracellular calcium concentration in response to testosterone provides the most robust evidence that androgens induce cellular effects through nongenomic signalling.³⁶ Hypogonadism is associated with an increased risk of osteopenia and osteoporosis, an effect normalized by testosterone replacement.³⁷ In male rat osteoblasts, low concentrations of testosterone, 0.1 to 1 nmol/L increase cytosolic free calcium and membrane phospholipid metabolism rapidly (5 to 60 seconds), followed by an increase in the cellular content of IP_3 and formation of diacylglycerol (DAG). These events are not observed in female rat osteoclasts, suggesting that this is a sex-dependent effect. Androgens can also activate L-type calcium channels, thereby increasing the intracellular levels of calcium, activating PKC, and via calmodulin, activating PKA and MAPK pathways.¹⁹

Nongenomic Ca^{2+} mobilization by androgens were also observed in murine macrophages. In macrophages, testosterone increases intracellular levels of Ca^{2+} .³⁸ During this process, androgens interact with membrane-associated AR, modulate G-protein activity, and subsequently activate PLC. This results in the rapid release of intracellular calcium stores from the sarcoplasmic reticulum and, consequently, activation of the RAS/MEK/ERK MAPK pathway.¹⁹ Likewise, through activation of plasma membrane AR associated with GPCR signalling in cardiac myocytes, stimulation with testosterone induces the release of Ca^{2+} from internal stores, such as endoplasmic reticulum and mitochondria.^{19,26}

Acute testosterone-induced nongenomic vasodilatation is mediated in part via endothelium-derived nitric oxide (NO).³⁹ Aortic endothelial cells (AECs) stimulated with testosterone or nonpermeable testosterone-BSA at physiological concentrations (1 to 100 nmol/L) present rapid (15 to 30 minutes) increases in NO level in AEC; testosterone also induces eNOS phosphorylation (Ser1177) without changing the total protein level. Activation of eNOS occurs via PI3K; caveolin-1; and c-Src binding to AR and, consequently, phosphorylation of AKT.²⁰ AR and s-Src mediate testosterone-induced rapid eNOS phosphorylation, as pretreatment with nilutamide or PP2, an AR and s-Src antagonist, respectively, abolishes the testosterone responses. Transcriptional inhibitor, actinomycin D does not affect testosterone-induced increase in NO, which excludes the classical genomic actions.⁴⁰ Anastrozole or other estrogen receptor antagonists do not interfere in NO generation induced by testosterone, suggesting that this is not an event associated with the aromatization of testosterone to oestradiol.⁴¹ In addition, testosterone at physiological concentrations inhibits $PGF2\alpha$ -induced Ca^{2+} fluxes by a nongenomic mechanism in vascular smooth muscle cells (VSMCs),⁴² which may contribute to testosterone-induced vasodilatation.

Testosterone-induced vasodilatation not associated with DNA-binding is also observed in humans. A recent study demonstrated that administration of transdermal-testosterone in men with hypogonadism and severe hypotestosteronemia causes acute vasodilatation and improves arterial stiffness by nongenomic mechanisms, although—interestingly—the improvement is also evident after 96 hours of treatment, which would suggest a combination of genomic and nongenomic effects to reach the same response.⁴³ In human aortic endothelial cells *in vivo*, testosterone can increase BK_{Ca} and SK_{Ca} currents, leading to rapid replorization and vasorelaxation via the action of a surgate androgen receptor, G_{i/o} protein, and protein kinase A.⁴⁴

Vascular Sex Differences in the Fetal Period

There are sex differences in the developmental programming of cardiovascular diseases. For example, in rats and guinea pigs, male pups subjected to perinatal hypoxia demonstrate greater susceptibility to the hypoxic insult than female pups.⁴⁵ Sexual dimorphism is implicated in physiological mechanisms for the development of cardiovascular disease.⁴⁶⁻⁵⁵ The underlying reasons why female and male subjects exhibit these differences have not yet been fully elucidated, and it is not clear whether androgens have any effects on these processes.

A newly described membrane protein, androgen-dependent tissue factor pathway inhibitor-regulating protein (ADTRP) has been identified as having potential implications for vascular development. This protein was first described, after the gene that encodes it—the C6orf105 gene—was found to be associated with coronary artery disease during genome-wide association studies of Chinese men with coronary artery disease.⁵⁶ This gene has since been associated with cardiovascular disease in several studies.⁵⁷ ADTRP itself is a membrane protein whose expression is upregulated by androgens and plays a major role in vascular development and function via expression in endothelial or perivascular cells of Wnt-regulated genes, which control vascular stability and integrity.⁵⁸

Vascular Sex Differences in Childhood

Sex differences in blood pressure have been described in children as young as 8 years of age, with boys being less likely to have a blood pressure within the normal reference range for age and height as defined using the age-specific American Heart Association blood pressure categories than girls (86.6% to 88.8% vs 93.0% to 96.3%).⁵⁹ Earlier onset of puberty and exposure to sex steroids have been shown to affect adult health outcomes, including cardiovascular health. One study of 3611 subjects using the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort found that systolic blood pressure at age 18 years reduced by 3.9 mm Hg per year of later puberty.⁶⁰ It is therefore essential that age-, height-, and sex-specific reference ranges are used in children for all clinical vascular phenotyping.

As discussed here, the prenatal surge in testosterone during the fetal period occurs during a critical time for normal masculinization of the genital tract.⁶¹ When there is

insufficient androgen during this period, genital anomalies—such as hypospadias—may develop. Hypospadias occurs in approximately 1 in 300 live births in Scotland⁶² and is defined as the abnormal positioning of the urethral meatus in boys. This condition therefore represents a model to investigate the effects of insufficient androgen exposure *in utero* on later vascular function. Vascular reactivity studies on resistance arteries from 46 XY boys with hypospadias and controls in early childhood (median age 18 months) demonstrated that arteries from boys with hypospadias had evidence of increased vasoconstriction vs controls and reduced endothelium-dependent and endothelium-independent vasorelaxation. This was in association with increased basal levels of reactive oxygen species (ROS) generation, hydrogen peroxide, and total antioxidant capacity in VSMCs from boys with hypospadias and activation of contractile mechanisms including rho-kinase activity and myosin light-chain phosphorylation. These findings suggest that androgen deficiency in the fetal period may alter critical determinants of vascular function in children. Within the same study, clinical vascular phenotyping of adolescents born with hypospadias demonstrated increased systolic blood pressure and carotid intima media thickness standard deviation score (SDS) as well as increased pulse pressure compared with controls and increased rates of admission to hospital for CVD in adults born with hypospadias.⁶³ Although it is not clear as to what the underlying mechanism for these changes is in this cohort, it is likely that androgen signalling is altered in this group, resulting in adverse cardiovascular outcomes.

Vascular Sex Differences in Adulthood

Hypertension is more common in men until the age of ~60 years, at which point women are more likely to be hypertensive.⁶⁴ Postmenopause, the average systolic blood pressure (SBP) of women is 5 mm Hg higher than that demonstrated pre- or perimenopause.⁶⁴ Altered estrogen : androgen ratios, activation of the renin-angiotensin-system (RAS), endothelin-1 (ET-1), sympathetic nervous system activation, increased inflammation, increased vasoconstrictor eicosanoids, and anxiety and depression have all been postulated as mechanisms for the increase in blood pressure seen in women postmenopause.^{65,66}

Atherosclerosis has also been demonstrated to present differently according to biological sex. Women are more likely to have diffuse atherosclerotic disease, with stable plaques that gradually erode, compared with men who often experience more acute plaque rupture, as recently reviewed by Man et al.⁶⁷ Differences in the development of atherosclerosis have been attributed to sex-specific gene regulatory networks involved in smooth muscle cell phenotype switching,⁶⁸ differences in vascular inflammation,⁶⁹ and sexually dimorphic body composition.⁷⁰ Differences in AR-mediated vascular remodelling, resulting in altered angiogenesis and increased risk of atherosclerosis, have been identified.⁷¹ In animal models, atherosclerosis and atherogenic lipids are increased in animals with an XX karyotype, regardless of hormone status, suggesting that there may be other mechanisms underpinning these differences outwith androgens and estrogens.⁷²

Specific Circumstances in Adulthood

Cardiovascular risk in androgen excess

Polycystic ovarian syndrome (PCOS)—a condition characterized by oligoanovulation, hyperandrogenism, and polycystic ovaries on ultrasound—is a useful clinical paradigm for the consideration of cardiovascular effects of androgen excess. Metabolic complications, including dyslipidemia and raised body mass index (BMI), are common in affected women, which may also exacerbate cardiovascular risk. Comparison of the cardiovascular events in patients with PCOS of reproductive age and in menopausal and aging women are higher than in healthy controls (pooled hazard ratio [HR], 1.38; 95% confidence interval [CI], 1.12-1.171 and 1.53; 95% CI, 1.15-2.04), respectively.⁷³ One small study of 151 Taiwanese women demonstrated that in women with PCOS, high testosterone levels, as measured by a raised free androgen index $\geq 19\%$ was associated with raised blood pressure (SBP ≥ 130 mm Hg \pm diastolic blood pressure [DBP] ≥ 85 mm Hg) (odds ratio [OR], 3.817; CI, 1.14-12.74; $P = 0.029$) independent of age, insulin resistance, obesity, or dyslipidemia.⁷⁴ However, a prospective study, including 8612 women from the Australian Longitudinal Study of Women's Health, suggests that although women with PCOS are more likely to be hypertensive than controls, and this is not associated with raised BMI,⁷⁵ again implying that high androgen levels play a greater role in the development of hypertension than other cardiovascular risk factors. Androgens seem to mediate this cardiovascular risk in PCOS via suppression of NO leading to reduced ET-1-induced vasodilatation both at the cellular level and demonstrated using Doppler flowmetry to assess microvascular endothelial function.⁷⁶

During pregnancy, androgens increase with gestation.⁷⁷ Pregnant women with PCOS have increased risk of pregnancy-induced hypertension, and it is not clear whether this is secondary to elevated androgen levels. In a study recruiting women with no previous history of PCOS, those with higher androgen levels in the third trimester were more likely to develop pre-eclampsia.⁷⁸ In addition, increased placental AR expression has been described in pre-eclampsia, as reviewed by Kumar et al.⁷⁹ As such, the role of androgens during pregnancy should also be an area for future research.

Another example of androgen excess can be seen in athletes who abuse anabolic steroids to increase muscle mass. Excess exogenous testosterone in this way has, however, been associated with an increase in cardiovascular-related deaths secondary to cardiac hypertrophy, thrombus formation, and arterial vasospasm.⁸⁰ Interestingly, however, the administration of testosterone supplementation to female-to-male transgender patients results in only modest increases in blood pressure, which resolve after cessation of therapy.⁸¹

Transgender persons receiving gender-affirming hormones should also be considered regarding the potential effects of intake of exogenous androgen. As reviewed by Connelly et al., there is some evidence that transgender persons may have increased risk of myocardial infarction, without associated increase in likelihood of angina or coronary artery disease, although data from studies remain conflicting.⁸² More recent reviews such as have demonstrated that whilst the risk of CVD remained low overall, transgender individuals were at

increased risk of CV events when receiving gender-affirming treatment compared with cisgender people, although the mechanisms for this remain unclear.⁸³

Cardiovascular risk in androgen deficiency

Androgen deficiency, or hypogonadism, is defined as low levels of circulating testosterone.

In animal models, androgen deprivation via orchietomy results in function and structural changes to the internal pudendal arteries, resulting in vascular endothelial dysfunction as well as reduced NO activity, which is reversed by replacement of testosterone.⁸⁴ In humans, studies analyzing the effects of androgen-deprivation therapy for prostate cancer have demonstrated increased risk of incident diabetes, myocardial infarction, CVD,⁸⁵ and vascular stiffness⁸⁶ across the range of different androgen-deprivation modalities including gonadotrophin-releasing hormone agonists, antagonists, androgen blockage, and CYP17 inhibitors, as discussed in a recent meta-analysis by Hu et al.⁸⁷

Basal testosterone levels are inversely related to mortality because of CVD in adult men.⁸⁸ All-cause mortality is increased in hypogonadal men and testosterone therapy reduces mortality to 8.4% compared with 19.2% in untreated groups.⁸⁹ A large French study ($n = 3650$) has identified a J-shaped association between total and bioavailable testosterone levels, as measured by mass spectroscopy, and CVD events with men in the lowest and highest total testosterone quintiles having increased risk.⁹⁰ In addition, some studies including a 4-year follow-up study found that progression of atherosclerosis is inversely associated with testosterone levels.^{91,92}

The mechanisms whereby hypogonadism exerts increased cardiovascular risk are likely multifactorial, caused by the many effects of testosterone on the vasculature. Hypogonadism has been associated with arrhythmia including long QT syndrome⁹³ and torsades de pointes.⁹⁴ In addition, hypertension has been associated with low testosterone levels in men undergoing investigation for erectile dysfunction,⁹⁵ and 25% of patients with heart failure are reported to have biochemical hypogonadism.⁹⁶ Testosterone can reduce body fat and regulate metabolism, which is also corroborated by the fact that adult men with hypogonadism have increased rates of metabolic syndrome.⁹⁷ Some key studies reporting risk of cardiovascular consequences in androgen deficiency (defined as circulating testosterone levels under the lower limit of the local reference range) in men are summarized in Table 1.^{90,98-109}

As noted, testosterone levels naturally decrease in men with aging. This effect has also been seen in women postmenopause, although other descriptions of women with androgen deficiency are scarce, as reviewed recently by Traish and Morgentaler.¹¹⁰ The cardiovascular benefits of testosterone replacement are controversial. Testosterone can reduce DBP and SBP in some cases but increase it in others, with no clear mechanisms underlying these differential effects.¹¹¹ Testosterone replacement in hypogonadal men has been demonstrated to reduce obesity, fat mass, waist circumference, and mortality as well as improve glycemic control and overall cardiometabolic status compared with placebo.¹¹² Other studies have demonstrated that testosterone can modulate the response of subcutaneous resistance arteries to vasoconstrictors

Table 1. Studies demonstrating a link between low endogenous testosterone level (hypogonadism) and cardiovascular disease

Reference	Number of patients	Outcome measure	HR (95% CI)	P
Zhang et al. ¹⁰⁹ (2021)	602	Framingham CV risk score	1.8 (1.64-2.12)	NA
Corona et al. ⁹⁸ (2011)	12,566	CVD diagnosis	2.55 (−3.39, −1.71)	< 0.0001
Corona et al. ⁹⁹ (2010)	1697	Major adverse cardiovascular event	7.1 (1.6-28.6)	< 0.001
Soisson et al. ⁹⁰ (2013)	3650	First ischemic arterial disease event	2.23 (1.02-4.88)	< 0.01
Farias et al. ¹⁰⁰ (2014)	115	CIMT	8.43 (2.5-25.8)	< 0.0001
Yeap et al. ¹⁰¹ (2009)	3433	Stroke	1.99 (1.33-2.99)	0.01
Laughlin et al. ¹⁰² (2008)	794	CV death	1.38 (1.02, 1.85)	NA
Araujo et al. ¹⁰³ (2011)	17,091	CV death	1.54 (1.28-1.85)	0.02
Malkin et al. ¹⁰⁸ (2010)	930	CV death	2.2 (1.4-3.6)	< 0.0001
Haring et al. ¹⁰⁴ (2010)	1954	CV death	2.56 (1.15-6.52)	< 0.05
Hyde et al. ¹⁰⁵ (2012)	3637	CV death	1.71 (1.12-2.62)	0.024
Pye et al. ¹⁰⁷ (2014)	2599	CV death	5.2 (2.0-13.7)	NA
Boden et al. ¹⁰⁶ (2020)	2118	CVD diagnosis	1.37 (1.1-1.7)	0.03

CI, confidence interval; CIMT, carotid intima media thickness; CV, cardiovascular; CVD, cardiovascular disease; HR, hazard ratio; NA, not available.

and vasodilators in different vascular beds, including rabbit coronary arteries and aorta; mouse iliac arteries; and rat coronary, mesenteric, and pulmonary arteries.¹¹³ Endothelial progenitor cells (EPCs) are essential for neovascularization, and androgens have been shown to increase EPC function, resulting in coronary collateralization in men with hypogonadism,¹¹⁴ as well as enhancing postischemic vascular repair by promoting angiogenesis.¹¹⁵

However, testosterone supplementation also increases hematocrit levels and reduces high-density lipoprotein (HDL) cholesterol levels, which can result in increased cardiovascular morbidity and mortality.¹¹⁶ Isolated clinical trials have suggested that testosterone replacement therapy (TRT) may result in adverse outcomes, but a recent meta-analysis of 39 randomized controlled trials and 10 observational studies, spanning trials of TRT of between 6 weeks' and 3 years' duration, and including 5451 men (3230 receiving TRT and 2221 placebo), demonstrated no significant association between testosterone therapy and major adverse cardiovascular events when testosterone was replaced to the normal healthy range.¹¹⁷ It is possible, therefore, that the excess in cardiovascular morbidity and mortality, seen in athletes who abuse testosterone, is secondary to the supraphysiological levels of testosterone ingested, which can be as high as 5 to 29 times greater than usual physiological replacement doses in men with previously normal androgen levels.¹¹⁸

Different routes of administration of testosterone may, however, be associated with variable cardiovascular risk,¹¹⁹ which may alter, depending on duration of treatment. The mechanisms underlying the differential effects of exogenous testosterone supplementation remain unclear. A summary of studies investigating the effects of testosterone replacement therapy on CVD has been published recently by Gagliano-Juca and colleagues.¹²⁰

Conclusions

The investigation of the effects of sex steroids on the vasculature is subject to a number of different confounders, making it a challenging field to be able truly to determine what the underlying molecular mechanisms are whereby sex hormones influence vascular function. As discussed, androgens have multiple actions on the vasculature, some of which are vasoprotective and others that induce vascular injury. Both

androgen excess and androgen deficiency can result in adverse cardiovascular outcomes, and there are many gaps in the scientific literature regarding the mechanisms for these differences—including the roles of sex steroid receptors and proteins such as AR, GPRC6A, and ADTRP—in these processes. The need to replace testosterone in adulthood has been of significant research interest, but it still remains unclear what the implications are of altered androgen signalling in early life, such as in children and young people with hypospadias. Understanding the physiological basis behind these changes may allow for the mitigation of cardiovascular risk later on.

In summary, although this review focuses on androgens, estrogens are also key players in the sexual dimorphism associated with vascular disease. The roles of the sex hormones may alter throughout the lifespan. Further research is however required to better understand the physiological basis behind these changes to allow for the mitigation of cardiovascular risk later on.

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