High Testosterone Levels: Impact on the Heart

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Implications for Testosterone Misuse

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Abstract

Testosterone, the main endogenous active androgen, is used to treat many clinical conditions, such as hypogonadism, infertility, erectile dysfunction, osteoporosis, anemia, and in transgender therapy (female-to-male transsexuals). Androgens are also used by athletes to enhance performance and endurance, and by nonathlete weightlifters or bodybuilders to enhance muscle development and strength. Accordingly, testosterone and other anabolic-androgenic steroids are the main class of appearance and performance enhancing drugs (APEDs), i.e., substances

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used to improve appearance by building muscle mass or to enhance athletic performance.

Testosterone and other androgens, mainly at supraphysiological levels, affect every single body tissue or system, including the cardiovascular system. Testosterone increases cardiovascular disease risk, causes myocardial infarction, stroke, high blood pressure, blood clots, and heart failure. Among the potential mechanisms whereby testosterone affects the cardiovascular system, both indirect and direct actions have been reported. Indirect actions of testosterone on the cardiovascular system include changes in the lipid profile, insulin sensitivity, and hemostatic mechanisms, modulation of the sympathetic nervous system and renin-angiotensin-aldosterone system. Direct actions of testosterone in the cardiovascular system involves activation of proinflammatory and redox processes, decreased nitric oxide (NO) bioavailability, and stimulation of vasoconstrictor signaling pathways.

This chapter focuses on the effects of androgens, mainly testosterone, on the vascular system. The effects of testosterone on endothelial and vascular smooth muscle cells, as well as mechanisms involved in the effects of testosterone will be reviewed. Effects of testosterone on the perivascular adipose tissue, the immune, sympathetic, and renin-angiotensin systems will also be mentioned.

Keywords

Androgen receptor · Cardiovascular · Contraction · Cyclooxygenase · Endothelial cell · Endothelium-derived factors · Nitric oxide · Relaxation · Testosterone · Vascular smooth muscle cell · Perivascular adipose tissue

Abbreviations

AAA	Ascending aortic aneurysms
ACh	Acetylcholine
Ang II	Angiotensin II
APEDs	Appearance and performance enhancing drugs
AR	Androgen receptor
ARKO	*AR* knockout mice
$AT_{1a}R$	Angiotensin II type 1A receptor
AT_2R	Angiotensin II type 2 receptor
Bcl-2	B cell leukemia/lymphoma-2
BK_{Ca}	Large-conductance Ca^{2+}-activated potassium channel
BSA	Bovine serum albumin
Ca^{2+}	Calcium ion
CASMCs	Coronary artery smooth muscle cells
Cav1.2	L-type voltage-gated Ca^{2+} channel
CDP	Collagenase-digestible protein
CDK	Cyclin-dependent kinase
CK	Creatine kinase
COX	Cyclooxygenase

CSMC	Coronary smooth muscle cell
DHEA-S	DHEA sulfate
DHEA	Dehydroepiandrosterone
DHT	5α-dihydrotestosterone
ECs	Endothelial cells
EDCFs	Endothelium-derived contracting factors
EDHF	Endothelium-derived hyperpolarizing factor
EDRFs	Endothelium-derived relaxing factors
EETs	Epoxyeicosatrienoic acids
eNOS	Endothelial nitric oxide synthase
ERK1/2	Extracellular signal-regulated kinase 1/2
Gas6	Growth arrest-specific gene 6
GPRC6A	GPCR, Class C, group 6, subtype A
H_2O_2	Hydrogen peroxide
HDL	High-density lipoprotein
HMG-CoA	3-hydroxy 3-methylglutaryl coenzyme A reductase enzyme
HUVEC	Human umbilical vein endothelial cell
iNOS	Inducible nitric oxide synthase
IL-1β	Interleukin-1beta
IP_3	Inositol trisphosphate
K^+	Potassium ion
Kcnn3	Small conductance calcium-activated potassium channel
Kv	Voltage-dependent potassium channel
LDL	Low-density lipoprotein
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
NAR	Normal androgen receptor
NCP	Noncollagen protein
NF-κB	Nuclear factor-κB
NLRP3	NOD-, LRR-, and pyrin domain-containing protein 3 inflammasome
NO	Nitric oxide
Nox1	Subtype 1 NADPH oxidase
Nox4	Subtype 4 NADPH oxidase
ORX	Orchiectomized
ORXT	ORX treated with testosterone
Ox-LDL	Oxidized low-density lipoprotein
PDGF	Platelet-derived growth factor
PGE_2	Prostaglandin E_2
$PGF_{2\alpha}$	Prostaglandin F_2 alpha
PGI_2	Prostacyclin
PKC	Protein kinase C
PTOV1	Prostate overexpressed protein 1
PVAT	Perivascular adipose tissue
RASMC	Rat aortic smooth muscle cell
ROS	Reactive oxygen species

SBP	Systolic blood pressure
SHR	Spontaneously hypertensive rat
siRNA	Small interfering RNA
SK3 channel	Small-conductance calcium-activated potassium channel-3
SMCs	Smooth muscle cells
T-BSA	Testosterone-3-carboxymethyl oxime conjugated to bovine serum albumin
TFM	Testicular feminized male
THG	Tetrahydrogestrinone
TNF-α	Tumor necrosis factor-alpha
TP	Thromboxane-prostanoid
TxA_2	Thromboxane A_2
VCAM-1	Vascular adhesion molecule 1
VSM	Vascular smooth muscle
VSMCs	Vascular smooth muscle cells
WKY	Wistar-Kyoto rat

Stating the Problem

Misuses or Non-prescribed Uses of Testosterone and Androgens

According to the National Institute of Health on Drug Abuse, testosterone and other anabolic-androgenic steroids are appearance and performance enhancing drugs (APEDs) (National Institute on Drug Abuse; Steroids and Other Appearance and Performance Enhancing Drugs (APEDs) Research Report).

APEDs are used to improve appearance by building muscle mass or to enhance athletic performance. Although APEDs do not produce euphory, as other abuse drugs do, APEDs users may develop a substance use disorder, i.e., they seek continued use despite adverse consequences or effects (National Institute on Drug Abuse; Steroids and Other Appearance and Performance Enhancing Drugs (APEDs) Research Report).

Androgens or anabolic-androgenic steroids represent the main class of APEDs. Androgens are any natural or synthetic compound that primarily influences the growth and development of the male reproductive system, including the activity of the accessory male sex organs and development of male secondary sex characteristics. Testosterone is the main endogenous active androgen, but other androgens include androstenedione, 5α-dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), and DHEA sulfate (DHEA-S). Synthetic androgens are steroid ester variations of endogenous androgens, mainly testosterone, and include testosterone cypionate, testosterone decanoate, undecanoate, enanthate, propionate, heptylate, caproate, phenylpropionate, isocaproate, and acetate (Tostes et al. 2016). Steroid precursors, such as tetrahydrogestrinone (THG) and androstenedione, are used to increase testosterone levels and are expected to produce similar effects as anabolic steroids. The

purchase and nonmedical use of these substances varies in different countries, being very often considered illegal (National Institute on Drug Abuse; Steroids and Other Appearance and Performance Enhancing Drugs (APEDs) Research Report).

Testosterone and other steroids are mainly used to treat hypogonadism (males with very low levels or no endogenous testosterone), infertility, erectile dysfunction, osteoporosis, and anemia. While very low doses are used to treat female androgen insufficiency-associated symptoms, high doses of testosterone are used in feminizing hormone therapy or transgender therapy (female-to-male transsexuals). As already mentioned, androgens are also used by athletes to enhance performance and endurance, which is considered doping, and by nonathlete weightlifters or bodybuilders to enhance muscle development and strength (Tostes et al. 2016).

Androgens affect mood and behavior, with effects ranging from increased user's confidence and strength, to increased aggressiveness. Androgens, mainly at supraphysiological levels, have many other effects, being associated with testicular and liver tumors, kidney failure, infections, hormonal unbalance [decreased sperm production, enlarged breasts, testicular atrophy, and hair loss (in men); voice deepening, decreased breast size, coarse skin, excessive body hair growth, acne, and male-pattern baldness (in women)], psychiatric disorders (aggression, mania, delusions, depression), and also cardiovascular events or increased cardiovascular disease risk, with reports of myocardial infarction, stroke, high blood pressure, blood clots, and heart failure (Bahrke et al. 1992; Urhausen et al. 2004; Santamarina et al. 2008; Palatini et al. 1996; Vanberg and Atar 2010; Bhasin et al. 2001; El Scheich et al. 2013; Pope et al. 2014; Tostes et al. 2016; Baggish et al. 2017).

Testosterone and the Cardiovascular System

Although both supraphysiological and subphysiological levels of testosterone are associated with increased cardiovascular risk, the effects of supraphysiological levels of testosterone have been surprisingly little studied. Several reports indicate that supraphysiological levels of testosterone affect the function and structure of the cardiovascular system.

Among the potential mechanisms whereby testosterone affects the cardiovascular system, both indirect and direct actions have been reported. Indirect actions of testosterone on the cardiovascular system include changes in the lipid profile [increasing low-density lipoprotein (LDL) levels and decreasing high-density lipoprotein (HDL) levels], insulin sensitivity, and hemostatic mechanisms, modulation of the sympathetic nervous system and renin-angiotensin-aldosterone system. Direct actions of testosterone in the cardiovascular system involves activation of proinflammatory and redox processes, decreased nitric oxide (NO) bioavailability, and stimulation of vasoconstrictor signaling pathways (Bahrke et al. 1992; Farhat et al. 1995; Hutchison et al. 1997; Bhasin et al. 2001; Urhausen et al. 2004; Palatini et al. 1996; Santamarina et al. 2008; Vanberg and Atar 2010; El Scheich et al. 2013; Herring et al. 2013; Pope et al. 2014; Tostes et al. 2016; Baggish et al. 2017).

Testosterone affects many cardiac functions and consequently myocardial performance. Testosterone affects calcium (Ca^{2+}) homeostasis, expression of alpha and beta adrenergic receptors, inotropic, chronotropic, and dromotropic responses, growth and hypertrophic responses, as recently reviewed (Pirompol et al. 2016; De Smet et al. 2017; Elagizi et al. 2018; Ribeiro Júnior et al. 2018; Bianchi 2018; Carbajal-García et al. 2020).

Testosterone also affects the vasculature by interfering with mechanisms that control vascular function (relaxation and contraction) and structure (plasticity, growth, and remodeling). Vascular function is mainly determined by factors released from nerve terminals (norepinephrine from the sympathetic nervous system), endothelial cells (ECs), cells from the perivascular adipose tissue (PVAT), from resident and infiltrated immune cells (cytokines and chemokines), blood cells (eosinophils, platelets), and by the intrinsic components of the vascular smooth muscle cells (VSMCs, ion channels, receptors, enzymes, protein exchangers, and structural proteins).

Here we focus on the effects of androgens, mainly testosterone, on the vascular system. The effects of testosterone on ECs and VSMCs will be discussed. Testosterone effects in the PVAT and other mechanisms that control vascular function will also be addressed. The mechanisms involved in the effects of testosterone in these cells and tissues will be reviewed (Fig. 1).

Vascular Effects of Testosterone

Impact of Testosterone on Endothelial Cells

The vascular endothelium is a monolayer of cells between the blood vessel lumen and VSMCs. Vascular ECs, a key cellular component of blood vessels, play important roles in vascular health and disease (Vanhoutte et al. 2017). Endothelial cells contain functional androgen receptor (AR) and are targets for androgens' action. As will be discussed, some androgen's effects rely on AR activation, while others occur independently of AR-molecular pathways (Nheu et al. 2011).

Since deprivation of androgen is associated with enhanced endothelium-dependent dilatation in adult men (Herman et al. 1997), it is expected that high androgen levels affect endothelial function. Indeed, testosterone affects the ECs by interfering with many signaling pathways. Testosterone enhances apoptosis induced by tumor necrosis factor-α (TNF-α) in cultured human ECs (Ling et al. 2002). Testosterone has been reported to either stimulate (McCrohon et al. 1999; Zhang et al. 2002) or inhibit (Mukherjee et al. 2002) expression of vascular adhesion molecule 1 (VCAM-1) through AR-mediated and AR-independent mechanisms. 5α-Dihydrotestosterone (DHT) increases expression of VCAM-1 in an AR-mediated manner and via a nuclear factor-κB (NF-κB)-dependent mechanism in human ECs (McCrohon et al. 1999; Death et al. 2004).

Testosterone influences the release and actions of endothelium-derived factors, including endothelium-derived relaxing factors (EDRFs) and endothelium-derived

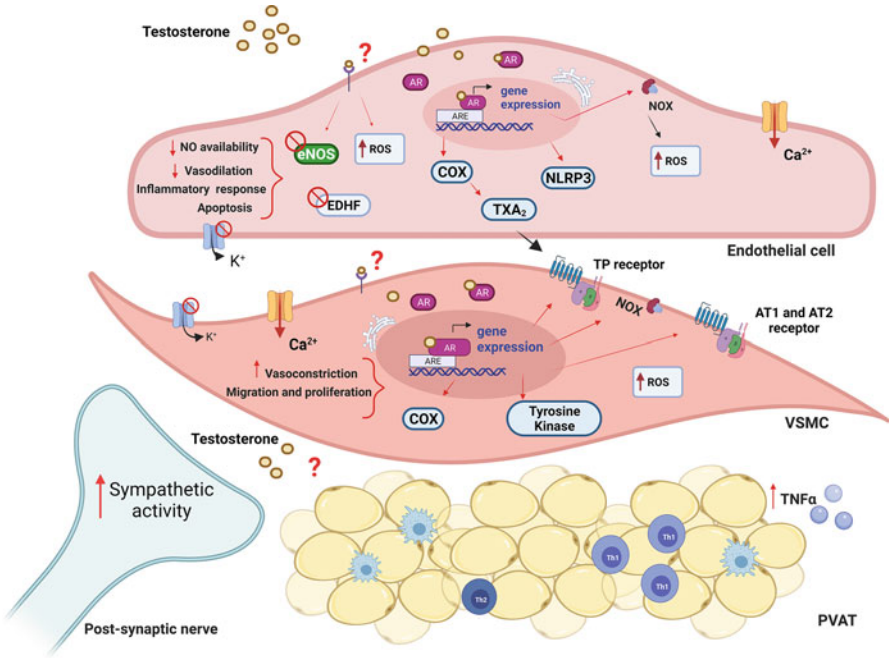

Fig. 1 Mechanisms whereby testosterone impacts vascular function. Vascular function is mainly determined by factors released by nerve terminals (norepinephrine from the sympathetic nervous system), endothelial cells, the perivascular adipose tissue, resident and infiltrated innate and adaptive immune cells (cytokines and chemokines), blood cells (histamine from mast cells; TXA_2 and serotonin from platelets), and by the intrinsic components of the vascular smooth muscle cells (ion channels, receptors, and structural and exchanger proteins). Testosterone affects the vasculature by interfering with all mechanisms that control vascular function. In the endothelium, testosterone modulates NO, COX-derived metabolites and EDHF release and signaling; in VSMCs, testosterone modulates ROS generation, expression, and activity of receptors and ion channels. AR: androgen receptor; ARE: androgen response element; TNF-α: tumor necrosis factor-alpha; NO: nitric oxide; eNOS: endothelial NO synthase; COX: cyclooxygenase; ROS: reactive oxygen species; NOX: NADPH oxidase; AT1 receptor: angiotensin II type-1 receptor; AT2 receptor: angiotensin II type-2 receptor; TP receptor: thromboxane-prostanoid receptors; TXA_2: thromboxane A_2; NLRP3: NOD-, LRR-, and pyrin domain-containing protein 3 inflammasome; EDHF: endothelium-derived hyperpolarizing factor; K^+: potassium ion; Ca^{2+}: calcium ion; Th1: T helper (Th) immune cells type 1; Th2: T helper (Th) immune cells type 2; VSMCs: vascular smooth muscle cells; PVAT: perivascular adipose tissue. "Created with BioRender.com"

contracting factors (EDCFs) (Farhat et al. 1995; Hutchison et al. 1997; Kumar et al. 2018). Testosterone impairs endothelium-dependent vasodilation through inhibition of the generation or signaling of EDRFs, including NO (Mäkinen et al. 2011), prostacyclin (PGI_2) (Nakao et al. 1981), and endothelium-derived hyperpolarizing factor (EDHF) (Gonzales et al. 2004).

Chinnathambi et al. (2013a, 2014a) found that elevated testosterone impaired acetylcholine (ACh)-, but not sodium nitroprusside (an exogenous NO donor)-induced relaxation in mesenteric and uterine arteries of rats, reinforcing that elevated

testosterone impairs endothelium-dependent relaxation. Specifically, decreased NO-mediated relaxations were reported in mesenteric and uterine arteries in a pregnant rat model with elevated testosterone (Chinnathambi et al. 2013a, 2014a). Testosterone-induced impairment of endothelium-dependent relaxations are due to decreased NO synthesis and release. In rats, testosterone reduces plasma levels of NO metabolites, a marker of NO bioavailability; it reduces endothelial NO synthase (eNOS) protein expression in uterine arteries (Chinnathambi et al. 2014a) and eNOS activity (increased phosphorylation at inhibitory Thr^{495} site and reduced phosphorylation at excitatory Ser^{1177} site) in mesenteric arteries (Chinnathambi et al. 2013a).

EDHF activity is represented by different substances in different vascular beds and different species (Campbell and Falck 2007). Moreover, gap junctions between ECs and VSMCs contribute to spread EDHF response along the vascular wall (Chaytor et al. 2001; Griffith 2004). Various substances have been proposed as mediators of EDHF activity, including potassium ion (K^+), C-type natriuretic peptide, hydrogen peroxide (H_2O_2), epoxyeicosatrienoic acids (EETs), and anandamide (Félétou and Vanhoutte 2006, 2009). Chinnathambi et al. (2014a) found that in addition to reduced eNOS protein, decreased mRNA expression of small-conductance Ca^{2+}-activated potassium channel-3 (SK3), which is a major source of EDHF-dependent hyperpolarization (Busse et al. 2002), occurs in uterine arteries of testosterone-treated rats. Impaired ACh-induced EDHF-mediated relaxation is also observed in these arteries. Female rats treated with DHT (7.5 mg, 90-day release) exhibit increased blood pressure and decreased EDHF-mediated relaxation in mesenteric arteries, an event associated with decreased connexin 43 expression (Mishra et al. 2017).

It is well established that hypertension has developmental origins, with exposure to adverse insults during the prenatal period causing the development of endothelial dysfunction and elevated blood pressure during adult life (Ligi et al. 2010). Prenatal exposure to elevated testosterone levels induces adult life hypertension associated with specifically impairment in EDHF-mediated relaxation in mesenteric arteries (Chinnathambi et al. 2013b; More et al. 2015). More et al. (2015) found that EDHF-type relaxation in testosterone-exposed offspring is decreased compared to that in controls, and it is improved by enalapril (an inhibitor of angiotensin converting enzyme) treatment. While *Kcnn4* channel expression and function are similar between control and testosterone rats, and it is not affected by enalapril treatment, *Kcnn3*-mediated relaxation is impaired in testosterone offspring, and it is normalized by enalapril treatment. In addition, enalapril treatment restores expression of *Kcnn3* channels.

Cyclooxygenase (COX)-derived prostanoids play an important role in the regulation of vascular tone and ECs are an important source of COX-derived metabolites (Félétou et al. 2011; Matsumoto et al. 2015; Imig 2020). Testosterone has been shown to modulate prostanoids signaling and responses. Testosterone increases the density of thromboxane-prostanoid (TP) receptors in platelets (Matsuda et al. 1994; Ajayi et al. 1995) and cultured rat aorta and canine coronary artery VSMCs (Matsuda et al. 1995; Higashiura et al. 1997). In aorta, coronary, and renal arteries, thromboxane mimetic U46619-induced contraction is increased by testosterone (Matsuda et al. 1994; Schrör

et al. 1994; Higashiura et al. 1996; Karanian and Ramwell 1996). TP receptors in vessels from males are more susceptible to testosterone modulation than those in female vessels (Matsuda et al. 1995; Karanian and Ramwell 1996; Higashiura et al. 1997). In rat cerebral arteries, chronic testosterone treatment enhances thromboxane A_2 (TxA_2)-mediated tone due to increased endothelial TxA_2 synthesis without changes in TP receptors-mediated contraction (Gonzales et al. 2005).

Increased testosterone alters uterine artery adaptations during pregnancy (Chinnathambi et al. 2014a, b). Thromboxane mimetic (U46619), phenylephrine, and angiotensin II (Ang II)-induced contractions are greater in endothelium-intact uterine arteries of testosterone-treated Sprague-Dawley rats (testosterone propionate; 0.5 mg/kg/day from gestation day 15 to 19) than in uterine arteries of controls (Chinnathambi et al. 2014a). Of note, enhancement of contractions induced by U46619 and phenylephrine by endothelial denudation is greater in controls than in testosterone-treated rats suggesting that the enhanced U46619- and phenylephrine-induced contractions of uterine arteries in testosterone-treated rats are mainly due to impaired endothelial function rather than increased U46619- and phenylephrine-induced contractions per se (Chinnathambi et al. 2014a). Indeed, EDRFs-mediated relaxations are impaired in uterine arteries of testosterone-treated rats. In contrast, treatment with testosterone augments Ang II-induced contractions in arteries with and without endothelium, suggesting that testosterone enhances arterial sensitivity to Ang II in VSMCs (Chinnathambi et al. 2014a), as will be discussed in the next section (Impact of testosterone on VSMCs).

For a summary of the impact of testosterone on vascular ECs, please refer to Table 1.

Impact of Testosterone on Vascular Smooth Muscle Cells

Not only ECs but also VSMCs contain functional AR and are targets for androgens actions. Biological activities of androgens in VSMCs are predominantly mediated through the AR, but many AR-independent effects also exist. AR-mediated effects involve transcriptional control of target genes while AR-independent effects usually involve androgens activation of multiple signaling pathways.

The effects of testosterone on all aspects of VSMCs function (tone, ion channels expression and activity, growth, and apoptosis) are also disputed and remains controversial (Barton et al. 2012; Campelo et al. 2012; Chignalia et al. 2012). However, whereas physiological levels of androgens and AR activation have been associated with both protective and deleterious effects on VSMCs function and remodeling, supraphysiological levels of androgens are practically always linked to vascular smooth muscle stress and harmful effects. Antagonic actions of testosterone and other androgens in the vascular smooth muscle may be related to local metabolism of testosterone by 5alpha-reductase type 2 and aromatase, enzymes responsible for conversion of testosterone into DHT and 17beta-estradiol, respectively, generating androgenic and estrogenic influences on blood vessels.

Table 1 Signaling pathways modulated by testosterone in endothelial cells

Endothelial cells				
Androgen	Animal/tissue	Molecular target	Impact on vascular function	Reference
Testosterone	Human intralobar pulmonary arteries		(+) Vasodilation (low concentration)	Rowell et al. 2009
			(+) Vasoconstriction (high concentration)	
Low levels of testosterone	Men (40–69 years) with andropausal symptoms		↓ Endothelium-dependent brachial artery flow-mediated dilatation	Mäkinen et al. 2011
Testosterone	Rat middle cerebral arteries	↓ EDHF	↓ Endothelium-dependent relaxation	Gonzales et al. 2004
Testosterone	Rat mesenteric arteries	↓ eNOS activity (↑ phosphor-Thr^{495} and ↓ phosphor-Ser^{1177})	↓ Endothelium-dependent and NO-mediated relaxation	Chinnathambi et al. 2013a, 2014a
Testosterone	Uterine arteries of testosterone-treated rats	↓ mRNA SK3 channel	↓ EDHF vasodilation	Chinnathambi et al. 2014a
DHT	Female rats treated with DHT	↓ Blood pressure	↓ EDHF-mediated relaxation of mesenteric arteries	Mishra et al. 2017
		↓ Connexin 43 expression in mesenteric arteries		
Testosterone	Prenatal exposure to elevated testosterone	↓ *Kcnn3*-mediated relaxation	Hypertension in adult life	Chinnathambi et al. 2013b
			↓ EDHF-mediated relaxation in mesenteric arteries	More et al. 2015
Testosterone cypionate	Healthy men	↑ TxA_2 receptor	↑ Platelet aggregation	Ajayi et al. 1995
Testosterone cypionate	Male rats	↑ TxA_2 receptor density in platelets and in aortic membranes	↑ Aortic contractile response to the TxA_2 mimetic, U-46619	Matsuda et al. 1994
DHT	Cultured male RASMC	↑ TxA_2 receptor density		Matsuda et al. 1995
Testosterone and DHT	Cultured RASMC and guinea pig CASMCs	↑ TxA_2 receptor density		Higashiura et al. 1997
		↑ TP receptors		

(continued)

Table 1 (continued)

Endothelial cells				
Androgen	Animal/tissue	Molecular target	Impact on vascular function	Reference
Antiandrogens flutamide or cyproterone acetate	Left anterior descending coronary artery, left circumflex coronary artery, and renal artery and vein of sexually mature dogs	Resulted in an increase in both the maximum	↓ Contractile response to the TxA_2-mimetic	Karanian and Ramwell 1996
Pretreatment of female dogs with testosterone			↑ Contractile response to U46619	
Testosterone	Isolated and perfused heart from male Guinea pigs		↑ U46619-induced pressor responses (contraction of coronary arteries)	Schrör et al. 1994
Testosterone	Cerebral arteries from rats on chronic testosterone treatment	↑ Endothelial TxA_2 synthesis	↑ TxA_2-mediated tone no changes in TP receptors-mediated contraction	Gonzales et al. 2005
Testosterone propionate	Endothelium-intact uterine arteries from testosterone-treated Sprague-Dawley rats		↑ U46619, phenylephrine, and Ang II-induced contractions	Chinnathambi et al. 2014a
			Causes endothelial dysfunction	
Androgen deprivation (bilateral orchidectomy and/or maximal androgen blockade for >6 months)	Adult men (40–70 years) under treatment of prostate cancer		↑ Endothelium-dependent dilatation	Herman et al. 1997
Testosterone	HUVEC stimulated with TNF-α		↑ VCAM-1 and E-selectin expression	Zhang et al. 2002
			↓ VCAM-1 expression (by testosterone conversion to	Mukherjee et al. 2002 estradiol by aromatase)
DHT	HUVEC	(+) Androgen receptor/NF-κB	↑ VCAM-1 expression	McCrohon et al. 1999
			↓ Human monocyte adhesion to human ECs	Death et al. 2004

(continued)

Table 1 (continued)

Endothelial cells				
Androgen	Animal/tissue	Molecular target	Impact on vascular function	Reference
Testosterone	HUVEC (EA.hy926) in culture	↓ Bcl-2 expression	↑ Cells in the early and late stages of apoptosis	Ling et al. 2002

Abbreviations: Ang II: angiotensin II; Bcl-2: B cell leukemia/lymphoma-2 protein; Ca^{2+}: calcium; CASMCs: coronary artery smooth muscle cells; DHT: 5α-dihydrotestosterone; EDHF: endothelium-derived hyperpolarizing factor; eNOS: endothelial nitric oxide synthase; HUVEC: human umbilical vein endothelial cell; Kcnn3: small conductance calcium-activated potassium channels; NF-κB: nuclear factor-κB; NO: nitric oxide; RASMC: rat aortic smooth muscle cell; SK3 channel: small-conductance calcium-activated potassium channel-3; TNF-α: tumor necrosis factor-alpha; TP: thromboxane-prostanoid; TxA_2: thromboxane A_2; VCAM-1: vascular adhesion molecule 1

Testosterone has major effects on vascular tone. Testosterone alters vascular tone through endothelium-dependent, as already discussed, and by endothelium-independent mechanisms. Testosterone affects many types of smooth muscle ion conductance channels, including K^+ and Ca^{2+} channels (Bowles et al. 2004; Jones et al. 2004; Scragg et al. 2004), receptors (angiotensin type 1 and type 2 receptors, adrenergic receptors, TP receptors), activity of enzymes including tyrosine kinase and NADPH oxidase, and redox system. For a summary of the impact of testosterone on vascular VSMCs, please refer to Table 2.

Expression of Ang II type 1b (AT_{1b}) receptor is increased, whereas Ang II type 2 (AT_2) receptor is reduced in testosterone-exposed arteries (Chinnathambi et al. 2014a). In adult male growth-restricted offspring, testosterone augments Ang II-mediated pressor responses (Ojeda et al. 2010). Moreover, contractile response to Ang II, but not to potassium chloride or phenylephrine, is increased in mesenteric arteries of testosterone-treated rats and increased maternal testosterone upregulates mesenteric vascular *Agtr1b* mRNA transcripts linked to an increase in blood pressure (Chinnathambi et al. 2014b). In general, AT_2 receptors are known to oppose AT_1 receptor-mediated responses, evoking vasorelaxation, natriuresis, antigrowth, and anti-inflammatory effects (Verdonk et al. 2012). Testosterone downregulates AT_2 receptor through androgen receptor-mediated extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathway in rat aorta (Mishra et al. 2016). Therefore, it is likely that the effect of testosterone on vascular smooth muscle contraction is agonist specific.

Proliferation and migration of VSMCs are key events in many pathological conditions such as hypertension, atherosclerosis, and restenosis (Ross 1999; Casscells 1992; Touyz et al. 2018). Testosterone and DHT stimulate proliferation of VSMCs through an AR-mediated mechanism (Fujimoto et al. 1994; Williams et al. 2002; Liu et al. 2003). In addition, testosterone exerts a mitogenic action in mammary artery smooth muscle cells (SMCs) by AR-independent mechanisms (Nheu et al. 2011). The involvement of a membrane-located AR, such as G protein-coupled receptor GPRC6A (GPCR, Class C, group 6, subtype A) (Pi et al. 2010; Cruz-Topete et al. 2020), in the control of VSMCs growth is likely since cell-impermeable bovine serum albumin (BSA)-linked testosterone has a growth effect in human vascular cells (Somjen et al. 2004).

Table 2 Signaling pathways modulated by testosterone in vascular smooth muscle cells

Vascular smooth muscle cell				
Androgen	Animal/tissue	Molecular target	Impact on vascular function	Reference
Testosterone	Cultured bovine aortic SMCs		↑ Incorporation of proline into CDP and NCP in the cell layer and medium	Leitman et al. 1984
Testosterone	Human aortic SMCs in culture	↓ HMG-CoA activity	↓ Cholesterol synthesis	Naseem and Heald 1987
Testosterone	Cultured rat aortic SMCs	↑ TxA_2 receptor density without any change in affinity		Masuda et al. 1991
		↑ Intracellular free Ca^{2+}		
		↑ IP_3 induced by U46619		
Testosterone	RASMC in culture	↓ PGI_2		Nakao et al. 1981
Testosterone and testosterone analogues (etiocholan-3 beta-ol-17-one, epiandrosterone, 17 beta-hydroxy-5 alpha-androst-1-en-3-one, androst-16-en-3-ol, and testosterone enanthate)	Rings of coronary artery and aorta of adult male or nonpregnant female New Zealand White rabbits	(+) K^+ channel	Vasodilation	Yue et al. 1995
Testosterone	Human aortic SMCs	↑ Cytosolic Ca^{2+} response to either LDL or Ox-LDL		Wells et al. 1996
Testosterone	Brachial artery in female-to-male transsexuals receiving long-term high-dose androgens		↓ Nitroglycerine-induced vasodilation ↓ Flow-mediated dilatation	McCredie et al. 1998

(continued)

Table 2 (continued)

Vascular smooth muscle cell				
Androgen	Animal/tissue	Molecular target	Impact on vascular function	Reference
Testosterone	Deendothelialized coronary artery strips isolated from castrated male pigs	↓ $^{45}Ca^{2+}$ influx	↓ Vascular contractions to $PGF_{2\alpha}$ and KCl	Crews and Khalil 1999
Testosterone	Porcine coronary artery rings		↓ Bradykinin and A23187 vasodilation	Quan et al. 1999
Testosterone	A7r5 smooth muscle cell line and HEK293 cells stably expressing either L- or T-type Ca^{2+} channels	(−) Both native and human recombinant vascular L-type Ca^{2+} channels	↓ Ca^{2+} influx into VSM and (+) vasodilation	Scragg et al. 2004
Testosterone DHT	Coronary arterial smooth muscle	↑ Cav1.2 expression and activity		Bowles et al. 2004
Testosterone	Isolated rat coronary arteries and thoracic aortae from control and AR-deficient testicular-feminized mice	Direct Ca^{2+} antagonistic action	Vasodilation	Jones et al. 2004
Androstenedione DHT Testosterone	Human VSMCs from internal mammary arteries	↑ Synthesis of biglycan and decorin	↑ Proliferation ↑ proteoglycan biosynthesis	Hashimura et al. 2005
Testosterone	Porcine CSMC in culture	↑ PKC expression and activity	↑ CSMC apoptosis ↓ Proliferation CSMCs	Bowles et al. 2007
		↓ ^{3}H-thymidine incorporation (+) G1 arrest		
		↓ S phase cells		
		↓ Cyclins D1, E		
		↑ p21(cip1) and p27(kip1)		
		↓ CDK2 and CDK6 activity		

Testosterone deprivation	Aortic artery and VSMCs in male Wistar rats	↓ Amplitude of Kv channel currents of aortic artery ↓ Kv1.5 expression in SMC	↓ Aortic constriction by 4-aminopyridine ↓ Kv function	Zhou et al. 2008
Castration	Sexually mature, male Yucatan miniature swine submitted to angioplasty (left anterior descending and left circumflex coronary arteries with balloon catheter overinflation and allowed to recover for 10 or 28 days)	↑ Intima-to-media ratio ↑ Intima-to-media normalized to rupture index	↑ Coronary neointima formation	Tharp et al. 2009
Testosterone DHT	Human aortic VSMCs	↑ AR-dependent transactivation of growth arrest-specific gene 6 (Gas6), ↓ P(i)-induced VSMCs apoptosis	↓ Vascular calcification	Son et al. 2010
Castration Testosterone	Testicular feminized male (AR deficient males) and normal androgen receptor (NAR) male rats	Second-order mesenteric artery responses to changes in intraluminal pressure (myogenic reactivity)	↓ SBP in both NAR and TFM ↓ Blood vessel reactivity (myogenic reactivity)	Toot et al. 2011
Testosterone	Neonatal testosterone administration (exposure of neonatal mice to testosterone)	↑ Abdominal aortic $AT_{1a}R$ mRNA abundance ↑ H_2O_2 generation in cultured abdominal aortic SMCs	↑ Ang II-induced atherosclerosis and AAA in adult females, but not in males	Zhang et al. 2012
Testosterone (short term)	Endothelium-denuded human umbilical artery	↑ mRNA expression of BK_{Ca} channel		Saldanha et al. 2013

(continued)

Table 2 (continued)

Vascular smooth muscle cell				
Androgen	Animal/tissue	Molecular target	Impact on vascular function	Reference
DHT (long term)		↓ Expression of the α-subunit of L-type Ca^{2+} channels		Saldanha et al. 2013
Castration	Male apolipoprotein E-deficient mice infused with Ang II for 1 month to induce AAA formation	↑ Smooth muscle α-actin and collagen	↓ Progressive dilation of suprarenal aortic lumen diameters during the continued Ang II infusion	Zhang et al. 2015
Testosterone DHT	Mouse VSMCs	↑ Phosphate (Pi)-induced calcification	(+) Vascular calcification through AR signaling	Zhu et al. 2016
		↑ Tissue non-specific alkaline phosphatase (Alpl) mRNA expression		
Castration	Male ARKO mice with vascular injury (ligation of the carotid artery).	↑ Outgrowth of VSMCs from ARKO	↑ Intimal area and intimal thickness (by endothelium-dependent mechanisms)	Wilhelmson et al. 2016
	Human aortic VSMCs	Castration ↓ cells migration and proliferation ↓ p27 mRNA and protein	AR deficiency in male mice ↑ Intimal hyperplasia in response to vascular injury	
Castration	Aorta of male and female Sprague-Dawley rats	(+) ERK1/2 MAP kinase-signaling	Castration ↑ AT_2R mRNA and protein	Mishra et al. 2016
DHT			↓ AT_2R in endothelium-intact but not endothelium-denuded aorta	Mishra et al. 2016
Testosterone	Cerebral blood vessels from male rats (intact, ORX, and ORXT)	↑ Vascular markers of inflammation, COX-2, iNOS ↑ Production of PGE_2	↑ Cerebrovascular inflammation both in vivo and in vitro	Razmara et al. 2005
	ECs and VSMCs after LPS treatment	In vitro LPS incubation ↑ COX-2 in pial vessels		

Testosterone DHT	Rat VSMCs	(+) DNA synthesis		Fujimoto et al. 1994
DHEA	Human VSMCs in culture	↓ ERK1 phosphorylation	↓ PDGF-induced increases in VSMCs proliferation	Williams et al. 2002
Androstenedione Testosterone			↑ VSMCs proliferation	
Testosterone DHT	Human mammary artery SMCs	↑ ERK1/2 activation and proliferation in SMC via AR-independent pathways	↑ Proliferation in SMC via AR-dependent mechanisms	Nheu et al. 2011
DHT T-BSA	Human VSMCs	↓ DNA synthesis ↑ MAPK activity ↑ CK activity	↓ VSMCs proliferation	Somjen et al. 2004
Castration	Adult male growth-restricted offspring		↓ Ang II-mediated pressor responses	Ojeda et al. 2010
Testosterone propionate	Pregnant Sprague-Dawley rats injected with testosterone (from gestational day 15 to 19)	↑ Plasma maternal testosterone ↑ Mesenteric vascular *Agtr1b* mRNA and protein expression	↑ Blood pressure ↑ Mesenteric artery contractions to Ang II	Chinnathambi et al. 2014
Testosterone	Human aortic VSMCs	↑ Expression of genes associated with cell proliferation ↑ Expression of human PTOV1	↑ Cell proliferation	Nakamura et al. 2006
Testosterone	Aortic VSMCs from SHR and WKY	↑ ROS generation ↑ Nox1 and Nox4 expression ↑ Phosphorylation of nonreceptor tyrosine kinase c-Src	↑ Migration of both WKY and SHR VSMCs	Chignalia et al. 2012

(continued)

Table 2 (continued)

Vascular smooth muscle cell				
Androgen	Animal/tissue	Molecular target	Impact on vascular function	Reference
Testosterone	Rat aortic VSMCs	↑ Mitochondria-generated ROS	Induces apoptosis in VSMCs	Lopes et al. 2014
Testosterone		↑ Nox and COX-2 activation	Induces leucocyte migration	Chignalia et al. 2015
Testosterone	Mouse arteries and VSMCs	↑ Mitochondrial ROS generation	Vascular dysfunction (increased contractions induced by phenylephrine and decreased ACh-induced relaxation)	Alves et al. 2020
		(+) NLRP3 inflammasome (↑ caspase-activation ↑ IL-1β release)		

Abbreviations: AAA: ascending aortic aneurysms; ACh: acetylcholine; Ang II: angiotensin II; AR: androgen receptor; ARKO: *AR* knockout mice; $AT_{1a}R$: angiotensin type 1A receptors; AT_2R: angiotensin II type-2 receptor; BK_{Ca}: large-conductance Ca^{2+}-activated potassium channel; Ca^{2+}: calcium ion; Cav1.2: L-type voltage-gated Ca^{2+} channel; CDP: collagenase-digestible protein; CDK: cyclin-dependent kinase; CK: creatine kinase; COX: cyclooxygenase; CSMC: coronary smooth muscle; DHEA: dehydroepiandrosterone; DHT: 5α-dihydrotestosterone; ERK1/2: extracellular signal-regulated kinase 1/2; Gas6: growth arrest-specific gene 6; H_2O_2: hydrogen peroxide; HMG-CoA: 3-hydroxy 3-methylglutaryl Co enzyme A reductase enzyme; IL-1β: interleukin-1beta; iNOS: inducible nitric oxide synthase; IP3: inositol trisphosphate; KCl: potassium chloride; Kv: voltage-dependent potassium channels; LDL: low-density lipoprotein; LPS: lipopolysaccharide; MAPK: mitogen-activated protein kinase; NCP: noncollagen protein; Nox1: subtype 1 NADPH oxidase; NAR: normal androgen receptor; Nox4: subtype 4 NADPH oxidase; ORX: orchiectomized; ORXT: ORX treated with testosterone; Ox-LDL: oxidized low-density lipoprotein; PDGF: platelet derived growth factor; PGE_2: prostaglandin E_2; $PGF_{2\alpha}$: prostaglandin F_2 alpha; PGI_2: prostacyclin; PKC: Protein kinase C; PTOV1: prostate overexpressed protein 1; RASMC: rat aortic smooth muscle cell; ROS: reactive oxygen species; SBP: systolic blood pressure; SHR: spontaneously hypertensive rat; SMCs: smooth muscle cells; T-BSA: testosterone-3-carboxymethyl oxime conjugated to bovine serum albumin; TFM: testicular feminized male; TxA_2: thromboxane A_2; VSM: vascular smooth muscle; VSMCs: vascular smooth muscle cells; WKY: Wistar-Kyoto rat

The detailed mechanisms underlying testosterone-induced VSMCs proliferation remain largely unknown. Using microarray and quantitative RT-PCR analyses, Nakamura et al. (2006) demonstrated that testosterone markedly induces the human prostate overexpressed protein 1 (*PTOV1*), which stimulates cell proliferation (Benedit et al. 2001) in VSMCs. Moreover, small interfering RNA (siRNA) analysis demonstrated that PTOV1 is involved in AR-mediated VSMCs proliferation. In human aorta obtained at autopsy, PTOV1, as well as AR, detected in the nuclei of neointimal VSMCs was abundant in relatively young male aorta at an early stage of atherosclerosis (Nakamura et al. 2006). Therefore, PTOV1 is considered to be one of the testosterone-induced genes associated with AR-mediated stimulation of VSMCs proliferation in the aortic neointima and may play key roles in androgen-related atherogenesis in the male human aorta.

Chignalia et al. (2012) demonstrated that testosterone affects VSMCs redox status and migration via genomic and nongenomic mechanisms, activating cellular events that may further aggravate hypertension-associated vascular dysfunction. Testosterone enhances reactive oxygen species (ROS) production in VSMCs from Wistar-Kyoto rat (WKY) and spontaneously hypertensive rat (SHR). In SHR, ROS production occurs at short- and long-term periods. Testosterone increases the expression of NADPH oxidase subunits (*Nox1* and *Nox4)* in WKY and SHR VSMCs. However, testosterone activates the nonreceptor tyrosine kinase c-Src with a similar time course for ROS formation only in SHRs. Thus, testosterone has a pivotal role in hypertension-associated processes, including vascular oxidative stress, activation of redox-sensitive pathways, and VSMCs migration and c-Src activation has a fundamental role in these effects.

Apoptosis deregulation is considered a pathogenic process in various diseases and is an important mechanism underlying the alterations seen in atherosclerosis, hypertension, and inflammation (Montezano and Touyz 2012). Testosterone has both pro- and antiapoptotic effects in different tissues/cells. In pig coronary VSMCs, testosterone treatment increases caspase-3 activity, an effect suppressed by protein kinase C (PKC)-δ siRNA (Bowles et al. 2007). Moreover, Lopes et al. (2014) demonstrated that testosterone induces apoptosis in VSMCs through the extrinsic apoptotic pathway with the involvement of AR activation and mitochondria-generated ROS. Testosterone may exert opposite effects on ROS generation and apoptosis, depending not only on the cell type but also on the cell functional status (Tostes et al. 2016). The complexity of testosterone effects is evident, and further studies are required for a better understanding of the pro- or anti-oxidative/apoptotic effects of testosterone, especially in the cardiovascular system.

For a summary of the impact of testosterone on VSMCs, please refer to Table 2.

Impact of Testosterone on the Perivascular Adipose Tissue Other Mechanisms That Control Vascular Function

Until recently, the adipose tissue was considered as only being involved in total body lipid and energy homeostasis. However, nowadays it is well known that adipose tissue also exerts major endocrine and paracrine effects via the release of various

inflammatory adipokines and other factors that affect vascular tone (Maenhaut and Van de Voorde 2011).

Initial studies suggested no effects of testosterone on the PVAT. Orchidectomy did not modify the anticontractile effects of PVAT of thoracic aorta male Swiss mice (Boydens et al. 2016). This study observed that 4 weeks of testosterone depletion by orchidectomy does not affect the protective effect of PVAT (Boydens et al. 2016). Since PVAT function changes in different stages of diseases, further investigation with time-course studies of PVAT's function in testosterone-depletion conditions are warranted. Testosterone injection reduces adiponectin levels in rodents (Nishizawa et al. 2002) and hypogonadal men have increased adiponectin levels compared with eugonadal subjects, which is decreased by testosterone replacement therapy (Lanfranco et al. 2004). These observations strongly suggest that testosterone impacts PVAT-derived adipokines, and further research may clarify the relationship among PVAT, testosterone, and vascular tone.

Although detailed molecular mechanisms underlying vascular dysfunction induced by supraphysiological testosterone remain unclear, inflammatory- or immune system-related events are also modulated by sex hormones, including testosterone (Roved et al. 2017; Shepherd et al. 2021).

Under physiological conditions, testosterone has direct effects on immune cells' function and in their inflammatory capacity (Shepherd et al. 2021). Testosterone is generally immunosuppressive while estrogen tends to enhance immune responses. Testosterone modulates immune cells activity, suppressing type 2 responses, i.e., responses mediated by Th2 cells, and increasing type 1 responses or Th1 cell-mediated responses (Roved et al. 2017).

Testosterone induces leucocyte migration through NADPH oxidase and COX-2 dependent mechanisms (Chignalia et al. 2015). Alves et al. (2020) recently demonstrated a link among supraphysiological testosterone levels, mitochondrial ROS generation, and NLRP3 inflammasome activation on vascular function. In vitro studies using thoracic aortic rings and in vivo protocols with wild-type and NLRP3 knockout mice demonstrated that testosterone activates the NLRP3 inflammasome in vascular cells, leading to vascular dysfunction, i.e., increased contractions induced by phenylephrine and decreased ACh-induced relaxation (Alves et al. 2020). Vascular dysfunction induced by testosterone involves NLRP3 inflammasome activation and oxidative stress, since testosterone-mediated vascular dysfunction is prevented by pharmacological inhibition of NLRP3 inflammasome, AR, and ROS generation (Alves et al. 2020). Testosterone also augments the expression of vascular markers of cellular inflammation, including COX-2 and inducible nitric oxide synthase (iNOS), and worsens cerebrovascular inflammation after intraperitoneal lipopolysaccharide (LPS) injection (Razmara et al. 2005). Since innate and adaptive immunity lead to vascular injury and are central in the pathophysiology of hypertension, heart failure, and other cardiovascular diseases, overactivation of the immune system may be central in the effects of supraphysiological levels of testosterone.

Increased sympathetic activity and autonomic imbalance are features in many cardiovascular diseases (Yoo and Fu 2020). Autonomic imbalance increases

peripheral vascular resistance, heart rate, and blood pressure. Increased sympathetic activity is also observed in healthy young men using androgens (Alves et al. 2010; dos Santos et al. 2013; Porello et al. 2018).

Testosterone also modulates norepinephrine levels and the activity of enzymes that control norepinephrine synthesis, i.e., tyrosine hydroxylase and dopamine-beta-hydroxylase. While castration decreases norepinephrine content and tyrosine hydroxylase activity, treatment of castrated animals with testosterone increases norepinephrine content and tyrosine hydroxylase and dopamine-beta-hydroxylase activity, suggesting that norepinephrine levels are under androgenic control (Müntzing 1971; Rastogi et al. 1977; Bustamante et al. 1989; Kumai et al. 1994).

Androgens increase vascular resistance and blood pressure in humans. In addition, increased resting muscle sympathetic nerve activity and lower forearm blood flow are observed in young men who use anabolic androgenic steroids. Androgens' users also exhibit increased heart rate during strength training and increased muscle sympathetic nerve activity and lower forearm blood flow in response to mental stress, indicating that androgens exacerbate neurovascular control throughout stress reactions (Alves et al. 2010; Porello et al. 2018). The increased blood pressure and augmented sympathetic outflow in androgens' users may increase cardiovascular disease risk in humans.

Applications to Other Areas of Public Health

Testosterone abuse, which has become increasingly popular in the last decades, may impose a substantial public health burden by increasing cardiovascular disease risk. Cardiovascular disease results in significant medical cost (for the individuals, private and government insurers, and other payers). Testosterone-induced cardiovascular disease (myocardial infarction, stroke, high blood pressure, blood clots, and heart failure – events reported with testosterone abuse) directly impact the individual quality of life.

Studies specifically addressing the cost burden imposed by consequences of testosterone abuse are scarce. However, since testosterone abuse is a potentially modifiable risk factor for cardiovascular disease, it may be responsible for substantial financial burden on the health care system and quality-of-life.

Key Facts of Testosterone

- Testosterone regulates many processes in the male and in the female body.
- Testosterone is used in clinical conditions (testosterone replacement therapy) and also in nonmedical conditions.
- Testosterone can carry cardiovascular effects and risks.
- Testosterone affects the vasculature by directly impacting endothelial and vascular smooth cells.

- Testosterone also changes vascular function by activating other systems (sympathetic, immune, renin-angiotensin).

Mini-Dictionary of Terms

Appearance and performance enhancing drugs (APEDs) are drugs used to improve appearance by building muscle mass or to enhance athletic performance.

Androgen is any natural or synthetic compound that primarily influences the growth and development of the male reproductive system, including the activity of the accessory male sex organs and development of male secondary sex characteristics.

Summary Points

- Testosterone, the main endogenous active androgen, is used to treat many clinical conditions.
- Testosterone and other androgens are also used by athletes, nonathlete weightlifters or bodybuilders to enhance muscle development, strength, and performance and endurance.
- Testosterone at supraphysiological levels increases cardiovascular disease risk, causes myocardial infarction, stroke, high blood pressure, blood clots, and heart failure.
- Testosterone affects the cardiovascular system by changing lipid profile, insulin sensitivity, hemostatic mechanisms, sympathetic nervous system, and renin-angiotensin-aldosterone system.
- Testosterone activates proinflammatory and redox processes, decreases nitric oxide bioavailability, and stimulates vasoconstrictor signaling pathways.
- Testosterone affects the vasculature by interfering with all mechanisms that control vascular function.
- In the endothelium, testosterone modulates NO, COX-derived metabolites and EDHF release and signaling.
- In VSMCs, testosterone modulates ROS generation, expression, and activity of receptors and ion channels.

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