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CARDIOVASCULAR AND METABOLIC ACTIONS OF THE ANDROGENS: IS TESTOSTERONE A JANUS-FACED MOLECULE?

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Abstract

Cardiovascular disease (CVD) is a major cause of morbidity and mortality worldwide and in the Western world, one-third of all deaths are attributed to CVD. A conspicuous characteristic of this healthcare epidemic is that most CVD is higher in men than in age-matched premenopausal women, yet reasons for these obvious sex differences remain poorly understood. Driven by clinical case and epidemiological studies and supported by animal experiments, a strong dogma emerged early on that testosterone (TES) exerts deleterious effects on cardiovascular health and exacerbates development of CVD and metabolic dysfunctions in men. In this review, earlier and more recent clinical and experimental animal evidence of cardiovascular and metabolic effects of androgens are discussed. The more recent evidence overwhelmingly suggests that it is progressive, age-dependent declines in TES levels in men that exacerbate CVD and metabolic dysfunctions, while TES exerts beneficial systemic hypotensive effects and protects against metabolic syndrome (MetS) and type2 diabetes mellitus (T2DM). Recent findings reveal existence of bi-directional modulation of glucose and fat homeostasis by TES in females *vs.* males, such that age-dependent declines in TES levels in males and abnormal increases in normally low TES levels in females both result in similar dysfunction in glucose and fat homeostasis, resulting in development of MetS and T2DM, central risk factors for development of CVD, in men as well as women. These findings suggest that the long-held view that TES is detrimental to male health should be discarded in favor of the view that, at least in men, TES is beneficial to cardiovascular and metabolic health.

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Key Words

Androgens

Androgen-Induced Vasodilation

Androgen-Induced Hypotension

Blood Pressure Regulation

Glucose and Fat Homeostasis

Hypertension

Sexual Dimorphism in Blood Pressure and Metabolism

Type 2 Diabetes Mellitus

Testosterone

ABBREVIATIONS

Androgen Deficiency	AD
Androgen Deprivation Therapy	ADT
Androgen Receptor	AR
Angiotensin Converting Enzyme	ACE
Angiotensin II Type 1 Receptor	AT_1R
Blood Pressure	BP
Calcium-Dependent, Large Conductance K^+ Channel	BKCa
Cardiovascular Disease	CVD
Congenital Adrenal Hyperplasia	CAH
Coronary Artery Disease	CAD
Dehydroepiandrosterone	DHEA
Deoxycorticosterone acetate-salt	DOCA-salt
5a-dihydrotestosterone	5α-DHT
5b-dihydrotestosterone	5β-DHT
17b-Estradiol	E2
Food and Drug Administration	FDA
Hypertension	HT
Hypothalamo-Pituitary Axis	HPA
Interleukin-6	IL-6
L-Type Voltage-Operated Ca^{2+} Channel	VOCC
Luteinizing Hormone	LH
Messenger Ribonucleic Acid	mRNA
Metabolic Syndrome	MetS
Neuronal Nitric Oxide Synthase	nNOS
Polycystic Ovary Syndrome	PCOS

Reverse Transcription-Polymerase Chain Reaction	RT-PCR
Sex Hormone Binding Globulin	SHBG
Sprague Dawley	SD
S-Methyl-Thiocitrulline	SMTC
Selective Androgen Receptor Modulator	SARM
Spontaneously Hypertensive Rat	SHR
Testicular-Feminized Male	TFM
Testosterone	TES
Testosterone Replacement Therapy	TRT
Tumor Necrosis Factor Alpha	TNFα
Type 2 Diabetes Mellitus	T2DM
Vascular Smooth Muscle	VSM
Voltage-Operated K^+ Channel	Kv
Wistar-Kyoto	WKY

1. Introduction

1.1. Androgen chemistry/structure-function

The sex steroid hormones are derived from the 27-carbon sterol precursor molecule cholesterol and are classified into one of three distinct families according to the number of carbon atoms contained; thus, there are the C18 estranes (estrogen derivatives), the C19 androstanes (androgen derivatives), and the C21 pregnanes (progesterone derivatives). The male sex steroid hormones (testosterone (TES) and related C19 androgens) are the molecules responsible for the differentiation, development, and maintenance of the male reproductive system and secondary sexual characteristics that so strikingly differ between males and females. In males, the biosynthesis of TES occurs in the Leydig (interstitial) cells beginning with the conversion of cholesterol to pregnenolone and then proceeding by one of two distinct routes, in which pregnenolone is first converted to the intermediate dehydroepiandrosterone (DHEA) and then to androstenediol via the Δ^5 pathway (predominant in humans) or alternately, in which pregnenolone is first converted to progesterone and then to androstenedione via the Δ^4 pathway (prevalent in other species). Both androstenediol and androstenedione then undergo direct conversion to TES, which may then be metabolized in some androgen target tissues to form the highly potent metabolite 5α-dihydrotestosterone (5α-DHT) or its largely inactive isomer 5β-DHT, or in peripheral tissues such as adipose to form 17β-estradiol (Fig. 1). In females, TES is produced in the theca cells of the ovarian follicles and is predominantly converted to 17β-estradiol in the neighboring follicular granulosa cells. It is important to emphasize that both androgens and estrogens are synthesized in both sexes and circulate in the plasma, albeit at quite different levels. Plasma levels of TES in females are 5-10% of that in males, while levels of estrogen in males are 10-30% of that in females; thus, androgens and estrogens play unique roles in the regulation of reproductive, cardiovascular, metabolic, and other functions in females and males [1,2].

1.2. History of androgens

TES was first identified as the principal mammalian sex steroid hormone of testicular origin in 1935; however, the role of the testis as the humoral source of male fertility and virility was recognized much earlier from the 18th century experiments of Hunter, which were subsequently replicated in the late 19th century by Berthold [3,4]. These experiments revealed that male secondary sexual characteristics and behavior must be dependent upon a humoral substance (identified much later as TES) from rooster testes that were transplanted into the abdominal cavity of castrated roosters. These studies and others provided an early conceptual recognition that the androgens might exert effects beyond those on the reproductive tract. Indeed, it is now recognized that the androgens exert a broad variety of physiologically relevant anabolic effects on bone and skeletal muscle, and contribute to the regulation of cardiovascular, hematopoietic, immune, metabolic, and nervous systems, with especially important roles in the regulation of the cardiovascular system and intermediary metabolism [1,2].

1.3. Mechanisms of androgen actions

The androgens may exert their effects on target tissues through both: (i) their well-known classic genomic actions, which are mediated through the cytosolic androgen receptor (AR) and require minutes to hours to achieve full effect, and (ii) their more recently identified non-genomic mechanisms of action, which are likely mediated through actions on the cell membrane and only require seconds to minutes to exert full effect.

The genomic mechanism of sex steroid hormone action was first identified in 1960 [5] and has been studied extensively for many years. Many if not all of the well-known biological effects of androgens on reproductive tract development and function, secondary male sexual characteristics and libido, anabolic actions on bone and skeletal muscle, and intermediary metabolism are known to be mediated by their genomic actions [6,7]. These classical effects of the androgens are known to be mediated through their binding to the cytosolic androgen receptor

(AR), which is expressed in many target tissues, with the highest concentrations in the male reproductive tract [8]. TES binding to the AR activates a genomic signaling pathway that involves binding of the androgen/AR complex to an androgen response element in the nuclear DNA, leading to the gene transcription and translation and subsequent changes in protein synthesis, which mediate the biological effects of TES on its target tissues (Fig. 2). This classical mechanism of AR-mediated androgen action is relatively slow and requires a time scale of minutes to hours to achieve full effect, and has been studied in detail (for reviews see [9-12]).

In contrast to the well-known classical genomic mechanism of action of TES and other sex steroid hormones, the non-genomic mechanism of sex steroid hormone action, which is independent of gene transcription and translation, was first identified in 1967 by Szego and Davis for estrogen [13], but much later for androgens [14]. These non-genomic mechanisms of action are more rapid, occurring within seconds to minutes, are independent of gene transcription, and have been observed in a variety of excitable tissues such as the central nervous system, reproductive and non-reproductive smooth muscle, as well as other tissues [15-17]. The non-genomic effects of androgens are mediated by a variety of intracellular signaling mechanisms including intracellular calcium and membrane binding proteins that can bind testosterone, such as the ZIP9 Zinc transporter or other membrane-associated receptor proteins such as the sex steroid binding globulin receptor. These receptors appear to mediate the effects of TES by rapid activation of membrane calcium channels, inhibitory G-proteins and the phospholipase C pathway, as well as ERK1/2, Akt, and CREB signaling cascades. Similarly, there is evidence that the androgens may interact directly with the cell membrane to alter ion channel function or with a membrane-associated G protein-coupled TES receptor that mediates AR-independent effects as well as modulates genomic effects of TES via ERK1/2/Akt pathways [15-17] (Fig. 2). One of these receptors has been associated with beneficial effects of TES in the heart via the PI3/Akt pathway [18]. Since nongenomic mechanisms of TES action appear to involve membrane receptors and intracellular signal transduction cascades, and these signaling mechanisms may also modulate

genomic expression, then it is also possible that a combination of genomic as well as nongenomic mechanisms may underlie the effects of androgens on cardiovascular and other systems.

1.4. The dogma of androgens in health and disease

Cardiovascular disease (CVD) is a major cause of morbidity and mortality worldwide, and in the Western World, one-third of all deaths are attributed to CVD [19]. One of the most conspicuous characteristics of this serious healthcare epidemic is that most forms of CVD are higher in men than in age-matched premenopausal women, yet the reasons for these obvious sex differences remain poorly understood. The clinical case studies and epidemiological observations that hypertension (HT) and coronary artery disease (CAD) occur more frequently in men than in premenopausal women [20-26] have led to the dogmatic view that TES and other androgens exert deleterious effects on the heart and vasculature and exacerbate the development of CVD in men, in part by worsening risk factors such as blood pressure, body fat composition, insulin resistance, and serum lipid profiles [27-29]. Indeed, the literature is replete with reports of the beneficial effects of estrogen on the female cardiovascular system (for reviews see [30-32]. In contrast, TES is usually considered to exacerbate CVD in males [20,27,28,33]. In parallel, most animal studies in the past have provided support for the dogmatic view that TES exacerbates CVD [34-38]. For example, studies in both genetic (Dahl salt-sensitive and spontaneously hypertensive) and induced (deoxycorticosterone acetate-salt [DOCA-salt]) rat models of HT reveal that castration ameliorates the development of HT in males [34,39,40]. However, more recent clinical and epidemiological studies on the role of TES in CVD are at best controversial, and in-depth reviews and analyses of the role of androgens in CVD reveal that there is little sound evidence that TES, or other androgens, shorten men's lives [24,26]. Indeed, more recent experimental animal studies [41-44] and human clinical trials [45-47] reveal that TES and other androgens exert beneficial effects on blood pressure and metabolic function, which are risk factors for CVD. Further, declines in TES levels with age or gonadal dysfunction are associated with deleterious effects on metabolic function, known as the metabolic syndrome (MetS), which

include dyslipidemia, hyperglycemia, insulin insensitivity, and development of type II diabetes mellitus (T2DM) [48-50].

Although plasma TES levels in females are only 5-10% of those in males, there is evidence that TES also plays a role in the regulation of metabolic profile, including fat distribution, glucose and lipid homeostasis, and insulin sensitivity, in parallel with the effects of estrogen on fat distribution. The sex-specific metabolic effects of androgens and estrogens result in clear sexual dimorphism in body fat distribution, adipose tissue function, and glucose homeostasis. Interestingly, pathological states in females associated with even modest elevations in androgen levels such as polycystic ovary syndrome (PCOS) result in the symptoms of MetS, including dyslipidemia, hyperglycemia, and insulin resistance [48-50].

The conundrum of dose- and sex-dependent effects of the androgens as beneficial *vs.* deleterious hormones important in the regulation of cardiovascular and metabolic function is reminiscent of the Roman God Janus who was the patron of doorways, beginnings and endings, passages, transitions, time, and duality, and was depicted as having two faces, symbolic of his ability to simultaneously look into the future and the past (Fig. 3). The two faces of Janus frequently symbolized change and transitions such as the progress of the past to the future, from one condition to another, from one vision to another, and represented time because he could see into both the past with one face, and into the future with the other. The related term Janus-faced then, seems to be an appropriate moniker for our current level of understanding of the conundrum of cardiovascular and metabolic effects of TES; thus, the present article will review the literature on cardiovascular and metabolic effects of TES and related androgens in males *vs.* females with the goal of solving the conundrum of beneficial *vs.* deleterious effects of TES and thereby answer the question: is TES a Janus-faced molecule?

2. Cardiovascular effects of the androgens

2.1. Historical perspective of the androgens

Long before TES was chemically identified as the principal mammalian sex steroid hormone in 1935 [51], the testes were recognized as the source of male fertility and virility. Indeed, the 18th century experiments of Hunter, replicated by Berthold in the 19th century, revealed from transplantation of testes into a castrated rooster that these organs were the source of a chemical that was responsible for the development of male secondary sexual characteristics and behavior [3,4]. The idea that the testes were the source of a substance responsible for male fertility and virility led Charles Edouard Brown-Sequard, a French physiologist and neurologist and pioneer in experimental medicine, to study (at the age of 72) the effects of self-injection of aqueous extracts of dog and guinea pig testes; he reported that these treatments restored his virility, vitality, and intellect for sustained periods [52]. Following the chemical identification of TES as a lipophilic steroid molecule in 1935, it became apparent that the rejuvenation that Brown-Sequard experienced from his organotherapy in 1889 was, in fact, merely a placebo effect, since his aqueous extracts of the testes could not possibly contain any TES. Nevertheless, the recognition that the testes were the source of beneficial effects on male vitality as well as their role in the maintenance of the reproductive tract, led to the wide use of TES and other androgens to treat a variety of clinical conditions and in human clinical and animal experiments during the next several decades.

2.2. The phases of androgen studies and clinical use in the cardiovascular system

Sarrel [53] identified three phases in the evolution of androgen use clinically and experimentally: 1) An early phase with great eagerness for the use of androgens in clinical conditions, especially CVD; 2) A subsequent period of disillusionment following recognition of the apparently deleterious side effects on cardiovascular health, based largely on human clinical and epidemiological studies, with further support from animal experimentation; and 3) A revisionist view of the role of androgens in cardiovascular health and disease, with renewed interest in

clinical use and re-examination of the problems with androgen use, with an emphasis on better design of experiments and data analyses.

A number of noteworthy clinical trials performed during the first phase of androgen use over 80 years ago first documented the beneficial effects of TES on the cardiovascular system. Acute intramuscular injections of TES were used successfully in the treatment of angina pectoris in men with CAD [54-56], and for hypertension and peripheral vascular disease [56,57]; these beneficial effects of TES were thought to involve vasodilation, although no direct measurements were made in these early studies. Interestingly, over the next three decades, numerous clinical and epidemiological studies focused on CAD and established its preponderance in men compared to women with a world-wide ratio of 2:1 (for review, see [20]). These studies identified the most important risk factors for this disease, including cigarette smoking, diabetes mellitus, hyperlipidemia, hypertension, and obesity, all of which are reversible; however, arguably the single most important factor is irreversible: that of male sex [58]. An extensive review of clinical studies over this time period by Kalin and Zumoff [20] came to the conclusion that "The most likely ultimate cause of the sex difference in the prevalence of coronary disease, and the one that has received the most attention, is the sex difference in sex hormone patterns" and that "the sex difference in the prevalence of coronary disease could as well be due to a "deleterious effect of maleness as to a protective effect of femaleness". They further concluded that "Androgens favor the development of coronary disease. This hypothesis is compatible with the preponderance of coronary disease in men, the decreased risk of coronary disease in hypotestosteronemic men with cirrhosis [which is accompanied by elevated estrogen levels], the increased coronary-disease risk in hypertestosteronemic women, and the increased coronary-disease risk in women taking androgenic synthetic progestogens." Such conclusions are doubtless what spawned the dogma that TES is deleterious to cardiovascular health and is largely responsible for the higher incidence of CVD in men than in women.

Indeed, the plethora of findings from epidemiological and clinical studies early in phase II led to the establishment of the dogma that TES has deleterious effects on the heart and vasculature and exacerbates development of HT and CVD in men [27-29,33]. This view was further supported by numerous experimental animal studies that provided overwhelming support for the dogma that the greater incidence of cardiovascular disease in men is due to the detrimental effects of TES on the heart and vasculature [34-38]. Despite the apparently overwhelming human clinical and experimental animal evidence that TES is detrimental, this view began to change with the emerging evidence that both androgens and estrogens exert rapid, non-genomic effects on the cardiovascular system that appear to be beneficial. These findings gradually led to a revisionist view in phase III that androgens may in fact exert beneficial effects, with renewed interest in their clinical use and re-examination of their effects on cardiovascular and metabolic disease. This revisionist view spawned an abundance of human clinical and experimental animal studies with an emphasis on better experimental designs and data analyses that have challenged the long-standing dogma surrounding the deleterious effects of androgens, and led to the new view that the androgens, at least at normal physiological levels, appear to be beneficial to human cardiovascular and metabolic health.

2.3. Cardiovascular effects of the androgens

Human clinical and experimental animal studies have clearly established that TES and other androgen metabolites exert beneficial effects by inducing relaxation of vascular smooth muscle (VSM) through rapid, non-genomic (androgen receptor (AR)-independent) mechanisms (for recent reviews, see [59-61]). Although this acute effect of TES and other androgens was initially reported at micromolar concentrations in large arteries (*e.g.,* aorta, coronary, mesenteric, radial, and umbilical) from a variety of animal species (dog, mouse, pig, rabbit, and rat) and humans, more recent studies have reported relaxation of smaller resistance arteries at nanomolar (physiological) concentrations (mesenteric, prostatic, pulmonary, and subcutaneous) (for reviews, see [59-61]. The key mechanism underlying this effect of TES on VSM appears to be activation

of calcium-dependent (BKCa) and voltage-operated (K_V) K^+ channels via TES-induced activation of VSM neuronal nitric oxide (nNOS) and/or inactivation of VSM L-type voltage-operated Ca^{2+} channels (VOCC) [60,61]. Several additional mechanisms may also contribute to TES-induced vasorelaxation of VSM at pharmacological (micomolar) concentrations, such as T-type Ca^{2+} channels and non-L-type VOCCs such as receptor-operated and store-operated Ca^{2+} channels. These mechanisms have been confirmed functionally in isolated blood vessels as well as electrophysiologically using patch-clamp methods in VSM cells [60]. An unequivocal determination of whether androgens activate K^+ channels or inactivate VOCCs and other Ca^{2+} channels is not possible at present, since most of the available studies have only explored one, but not both of these possible mechanisms. Indeed, androgens may both inactivate inward Ca^{2+} currents carried by VOCCs and activate outward K^+ currents carried by K^+ channels in VSM cells; however, a definitive answer will require more comprehensive studies that examine the roles of both mechanisms simultaneously. In the meanwhile, the reader is referred to a review that has examined this controversy in detail [62].

Although numerous studies have clearly established the rapid, nongenomic vasorelaxing effects of TES and other androgens *in vitro*, evidence that TES produces coronary or systemic vasodilation *in vivo* at physiological concentrations (100 pM to 100 nM) is limited. Indeed, intra-arterial infusion of TES produces coronary vasodilation in anesthetized dogs [63], pigs [64], and humans [65], and regional vasodilation of mesenteric, renal, and skeletal muscle vascular beds in anesthetized pigs [64]. All of these studies employed intra-arterial infusions of TES that were estimated to produce physiological plasma concentrations, and most resulted in strikingly similar levels of vasodilation (8-15%). More recently, several studies have demonstrated that *i.v.* infusions of TES and other androgens produce systemic hypotension in conscious rats *in vivo*. The first of these studies employed conscious, ganglionic-blocked male SD rats, which revealed that both TES and its genomically inactive metabolite 5β-DHT produced dose-dependent systemic hypotension. TES reduced mean arterial BP by a maximum of 16%, strikingly similar

and fully consistent with the local vasodilatory action of TES reported in previous studies above, while 5β-DHT was more efficacious than TES, and reduced BP by 23% [41]. Interestingly, infusions of TES in Testicular-Feminized male rats (Tfm; AR-deficient) in the same study reduced BP to the same extent as AR-intact SD rats. Pretreatment of SD rats with the nNOS inhibitor S-methyl thiocitrulline (SMTC) abolished the hypotensive effects of TES on BP. These findings suggest that the acute systemic hypotensive effects of TES and other androgens involve a direct vasodilatory action on the systemic vasculature which, like the effects observed in isolated arteries *in vitro*, is structurally specific and AR-independent, and involves activation of nNOS. Similarly, bolus *i.v.* injections of TES, 5α-DHT, and 5β-DHT in Spontaneously Hypertensive (SHR) and normotensive-control WKY rats produced dose-dependent systemic hypotension, with markedly greater hypotensive effects in SHR, and a rank order potency of 5β-DHT > TES > 5α-DHT [42]. (Table 1).

While these recent studies clearly establish that exogenous TES and other androgens exert important hypotensive effects on systemic BP through direct vasodilatory actions on the systemic vasculature, the question of the role of endogenous androgens in the long-term regulation of BP remained unanswered until recently. Long-term studies by Perusquia *et al* [43] revealed that castration of both Wistar and WKY male rats results in the progressive development of hypertension from a baseline of 110±2 mmHg to a maximum of 151±2 mmHg mean arterial BP at 11 weeks, that then plateaued through 18 weeks. Acute *i.v.* bolus injections of DHEA, TES, and 5β-DHT in the Wistar rats at 18 weeks produced dose-dependent reductions in BP with a rank order of potency of 5β-DHT = DHEA > TES. Subsequent long-term studies by Hanson *et al* [44] demonstrated that castration of male SD rats results in progressive hypertension from a baseline of 109±3 mmHg to a maximum of 143±3 mmHg systolic BP at week 10 post-castration, and that subsequent TES replacement therapy to physiological levels with TES-enanthate completely normalized BP in 5 weeks to 113±1.3 mmHg. Interestingly, castration and subsequent

TES replacement therapy of Tfm rats results in a similar pattern of hypertension, but with a more rapid normalization of BP with TES therapy to a level slightly lower than that of SD male rats (106±3 mmHg). Treatment of castrated SD male rats with the angiotensin receptor antagonist Losartan completely prevented the development of hypertension, and rt-PCR of the kidney revealed that castration increased expression of mRNA for renin (92%), angiotensin converting enzyme (ACE; 58%) and angiotensin II type 1 receptor (AT_1R; 80%) compared to intact control rats, while TES replacement completely normalized expression of renin, ACE, and AT_1R mRNA to levels of intact control rats. Plasma renin levels exhibited changes parallel to those of renin mRNA expression. These findings reveal that both endogenous and exogenous TES exert anti-hypertensive effects that appear to involve non-genomic and possibly genomic mechanism(s), resulting in reductions in RAS expression in the kidney, enhanced fluid and sodium excretion, and enhanced systemic vasodilation.

2.4. Sex differences in the cardiovascular effects of the androgens

Perhaps not surprisingly, the overwhelming majority of studies on the cardiovascular effects of the androgens in experimental animals, and to a lesser extent in human clinical studies, have employed males. However, given that measurable levels of TES and other androgens are present in the circulation of females, then it is also important to study the effects of these hormones in females. Studies of the acute effects of TES on blood vessels isolated from females, while limited, have uniformly revealed that TES-induced vasorelaxation in rat aorta, human pulmonary artery and vein, and isolated, perfused human lung do not differ between males and females [66-68]. Similarly, acute intra-arterial infusions of TES in anesthetized pigs produced similar regional vasodilation in females as well as males [64]. In normal-pregnant and preeclamptic-pregnant female Wistar rats near term, bolus *i.v.* injections of DHEA, TES, 5α- and 5β-DHT produced substantial reductions in mean arterial BP, with DHEA and 5β-DHT exhibiting significantly greater hypotensive potency than TES or 5α-DHT [69]. Isolated thoracic aortae from these same pregnant female groups exhibited similar vasorelaxing responses to these androgens. Several

studies have examined the effects of long-term TES treatment on vascular function and BP in animals and humans. Thus, long-term treatment of female Cynomologous monkeys with TES while fed a high fat diet increased coronary arterial atherosclerotic plaques but improved vasomotor responses to acetylcholine [70]. Similarly, long-term treatment of ovariectomized female SHR rats with TES for 5-10 weeks increased arterial BP to levels similar to those of male SHR [34,71]. In humans, long-term high dose TES treatment of female-to-male transexuals impaired flow-mediated vasodilation [72]. Several prospective human clinical studies identified positive relationships between plasma free (bioavailable) TES levels and the incidences of HT and CAD [73,74], and in postmenopausal women, higher serum levels of total and bioavailable TES and lower levels of sex hormone binding globulin (SHBG) were associated with a greater risk of HT and higher increases in BP [75].

2.5. Androgen chemical structure-function relationships

The numerous *in vitro* blood vessel and more recent *in vivo* BP studies of TES and other androgens reveal that the non-genomic vasodilatory effect is a structurally specific effect of the androgen molecule, which exhibits a structure-function relationship fundamentally different from that of its well-known AR-mediated genomic effects in reproductive tract target tissues. Of particular interest is the substantial vasodilatory efficacy of the major nonpolar excretory metabolites androsterone and etiocholanolone, which are virtually devoid of genomic effects in reproductive tissues, and the significantly lower vasodilatory efficacy and potency of 5α-DHT [76], which exhibits at least twofold greater efficacy and potency than TES in its genomic effects in reproductive tissues [77,78]. These findings establish that the relative polarity of the androgen molecule is a primary determinant of its vasodilatory efficacy and/or potency. The esterified TES analogs TES-enanthate and TES-hemisuccinate, which are substantially less polar (and, therefore, more lipid soluble) than TES, exhibit significantly lower efficacy and potency for vasodilation than does TES. Thus it has been proposed that TES-induced vasodilation is a structurally specific effect of the TES molecule, which is enhanced in more polar analogs that

have a lower permeability to the VSM cell membrane. This idea is supported by experiments with TES-hemisuccinate, a nonpolar esterified analog with high permeability to the VSM cell membrane and therefore relatively low vasodilatory efficacy. Conjugation of this TES analog to bovine serum albumin, which eliminates the permeability of this analog to the VSM cell membrane, dramatically enhances the both the efficacy and potency of this analog to cause vasodilation [76].

Another fascinating aspect of the structural specificity of androgen effects on vascular function is the peripheral metabolism of TES to its 5-reduced dihydro-metabolites (5α and 5β reductions), which include 5α-DHT via the enzyme 5α-reductase type 1 and type 2 and 5β-DHT via the enzyme 5β-reductase. While 5α-DHT exhibits a lower efficacy and/or potency than TES to produce vasorelaxation in virtually all blood vessels studied to date, its stereoisomer 5β-DHT is notably more efficacious and/or potent than TES to produce vasorelaxation. In contrast to its 5α-DHT stereoisomer, the 5β-DHT metabolite is virtually devoid of androgenic activity in reproductive target tissues. While the circulating plasma concentrations of these TES metabolites are much lower than that of TES, it is important to recognize that the levels of 5α- and 5β-DHT in androgen target tissues that express 5α- and 5β-reductase are likely to be much higher, which suggests that these metabolites act mainly as intracrine mediators in the androgen target tissues in which they are formed, such as the prostate gland, in which tissue concentrations of 5α-DHT are 10-fold higher than those of plasma. Thus, the same may be true for 5β-DHT in the vascular wall. Structurally, the A-ring of the steroid nucleus is planar in the TES molecule and in the α/trans configuration at C_5 of reduced metabolites such as 5α-DHT. In contrast, the A-ring is folded 90° relative to the steroid nucleus when the C_5 hydrogen is β/cis oriented, as is the case in 5β-reduced androgens such as 5β-DHT (Fig. 4). Clearly, the structural change in the 5β configuration is critical for the enhanced vasodilatory efficacy of 5β-DHT reported in *in vitro* as well as *in vivo* studies and this structural change may then also relate to its permeability in the VSM cell

membrane (for reviews, see [60,76]). It is of interest then that 5β-DHT exerts potent hypotensive effects *in vivo* in both normotensive and hypertensive rats [42,60,69] and that the activity of 5β-reductase is significantly lower in patients with essential hypertension compared with their normotensive counterparts [79]. Thus, 5β-reduced androgens such as 5β-DHT produced in the vascular wall may play a role in BP regulation by reducing vascular tone.

2.6. Significance of the cardiovascular effects of the androgens

There is a firmly entrenched dogma surrounding CVD that estrogens are protective in females [30-32], whereas androgens are deleterious in males [27,28,33]. These views appear to be based largely on earlier epidemiological data that have driven human clinical and experimental animal studies which appear to support the dogmatic view that TES is deleterious to the heart and vasculature [27-29]; however, many of those past animal as well as human studies suffer from flaws or limitations in experimental design and/or the animal models employed [24,26,45]. More recently published clinical and experimental animal studies challenge the TES dogma. Indeed, there is increasing evidence that TES therapy in aging hypogonadal men significantly improves cardiovascular and metabolic functions, including a reduction in diastolic blood pressure [45,80,81]. Further, plasma TES levels are reduced in both hypertensive men and women compared to their normotensive counterparts [22,82-84]. It is difficult to reconcile the enigma of beneficial effects of TES reported in more recent human clinical studies with the many past experimental animal studies which support the dogma of deleterious effects of TES [34-38], unless more careful scrutiny is applied to these studies. Thus, widely used genetic and induced rat models of HT, such as the spontaneously hypertensive rat (SHR) and Dahl salt-sensitive rat [34,40] and the DOCA-salt rat [39], respectively, have uniformly demonstrated that TES does exacerbate HT in males, since castration of males reduces the development of HT. However, these models do so in an unrealistic experimental setting that does not mimic human hypertensive disease, since the effects of TES are determined on a background of established HT (genetic models) or simultaneously developing HT (induced models), rather than on a background of

normotension, followed by progressive, age-dependent declines in TES levels and subsequent development of HT, as occurs in human males. Further, the short-term nature of these experiments (often 1-4 weeks) is problematic to the detection of longer-term effects of TES on BP in otherwise normal male rats. These limitations may explain why findings from these established animal models of HT are so incongruous with recent clinical findings from aging human males in which hypogonadism is associated with cardiovascular and metabolic dysfunctions, including HT, and that TES therapy is beneficial in reversing these conditions. Perhaps even more surprising is that normal male SD and Wistar rats, like men, do in fact exhibit age-dependent declines in TES [85,86], and male Fisher-344, SD, and Wistar rats all exhibit age-dependent increases in BP [87-89], similar to human males, yet this relationship has never been studied long-term in an animal model until recent studies in the laboratories of Perusquia [43] and Stallone [44].

A similar incongruency exists in the findings from human clinical studies. Because of the increasing marketing and use of TES replacement therapy in older men over the last ten years, a number of clinical trials have been performed. Some of these studies purport to show serious detrimental effects of TES on cardiovascular health and were widely publicized, likely because of the dogma surrounding the detrimental effects of TES, adding to the controversy. However, in both the prospective study of Basaria *et al*. [90] and the retrospective study of Vigen *et al.* [91], subsequent analyses of these studies revealed serious validity issues with experimental design (including TES doses), data collection and analysis, and selective exclusion of data that led the FDA to question validity of both studies. (for review see [46]). These examples reveal the importance of careful study design and that dogma and controversy can adversely distort the validity of human clinical findings concerning TES. From a larger perspective, of nine recently reported meta-analyses, all but one demonstrated that TRT is not harmful and is associated with significant health benefits [46,47]; thus, providing strong and valid support for beneficial effects of TES on the cardiovascular system.

The most recent human clinical trials overwhelmingly report that TRT does not increase cardiovascular risk or mortality in older hypogonadal men and that such therapy is associated with reductions in BP and improvement in MetS (*e.g.,* insulin resistance, serum lipids, diabetes, etc.), all of which are risk factors for CVD (for reviews see [45-47]. Indeed, the findings of recent experimental animal studies [41-44,69] are entirely consistent with these recent human clinical trials, and with much earlier studies in which acute intramuscular injections of TES were used successfully to treat angina pectoris in men with CAD [54-56], and for HT and peripheral vascular disease [56,75], and were thought to involve vasodilation, and with more recent clinical studies in which intra-arterial infusions of TES produced coronary vasodilation [65,92,93]. Taken together, these past and present studies strongly suggest that TES and other androgens are, in reality, anti-hypertensive and improve metabolic risk factors, and thus are protective against CVD, and it is the progressive age-dependent declines in plasma TES levels that exacerbate CVD in men.

3. Metabolic effects of androgens

3.1. Historical perspective of metabolic effects of the androgens

As discussed earlier in this article, the early 18th and 19th century experiments of Hunter, Berthold, Brown-Sequard, and others lead to the recognition that the testes were the source of a humoral substance(s) with beneficial effects not only on the function of the reproductive tract, but also on overall vitality, physical strength, and intellectual capacity, long before the chemical identification of TES as the humoral agent that mediated these effects. Once TES was chemically identified and synthesized in 1935, this subsequently led to the wide use of TES and other androgens to treat a variety of clinical conditions and in human clinical trials and animal experiments during the next several decades. Two key areas of study and clinical investigation that continue to this day are the effects on cardiovascular and metabolic health and disease. As discussed above, earlier human epidemiological and clinical trials led to the strongly entrenched dogma that androgens exacerbate CVD in men and male animals. An extensive review of these

earlier studies on sex steroids and CVD led Kalin and Zumoff [20] to propose the hypothesis that: "Androgens favor the development of coronary disease. This hypothesis is compatible with the preponderance of coronary disease in men, the decreased risk of coronary disease in hypotestosteronemic men with cirrhosis [which is accompanied by elevated estrogen levels], the increased coronary-risk in hypertestosteronemic women, and the increased coronary disease risk in women taking androgenic synthetic progestogens." They also implied that androgens have direct detrimental effects on the atherogenic process in the coronary arteries. This view reflects the long-known major influence of the androgens on body fat composition, muscle mass, and bone density in the male [94,95] and the idea that these hormones also exerted detrimental effects on lipid metabolism (*e.g.,* dyslipidemia), an important risk factor in CVD. This belief was advanced further by reports that athletes using anabolic steroids (synthetic derivatives of TES) to enhance physical strength exhibited premature, higher incidences of HT, ventricular remodeling, and sudden cardiac death [96-98]. However, more recent human epidemiological and clinical trials have challenged this idea and reveal that in addition to the well-known classical effects of TES on muscle mass and bone density, that it also plays a key beneficial role in the regulation of carbohydrate, fat, and protein metabolism and inflammation, and that TES deficiency is strongly associated with increased fat mass, elevated plasma triglycerides and total cholesterol, hyperglycemia, and insulin resistance, as well as elevated BP, the cluster of symptoms comprising the MetS and T2DM. MetS and T2DM share a commone etiology (central adiposity) and are central risk factors for CVD (for reviews, see [94,95,99].

3.2. Role of androgens in regulation of metabolism in males in health and disease

The recognition that androgens play a central role in the regulation of intermediary metabolism in health has largely been derived from clinical studies of androgen deficiency (AD) in men and its associated metabolic consequences. Male AD arises from failure of testicular TES production, either in the context of primary testicular pathology (primary male hypogonadism) or

hypothalamic-pituitary disease (secondary male hypogonadism). Acquired male hypogonadism may result from lesions or tumors of the testis or central nervous system, radio- and chemotherapy, pharmacological or surgical treatment, or chronic illness/poor health. Interestingly, male AD also frequently arises from normal aging or obesity and many recent studies have emphasized the link between hypogonadism, MetS, T2DM, and CVD (for reviews, see [94,50]. Indeed, the impact of TES deficiency on the development of visceral obesity, insulin resistance, and MetS in men is well established (for review, see [48]). Human clinical and experimental animal studies have identified several key actions of TES in the male that promote glucose and lipid homeostasis, including: prevention of visceral fat accumulation (adipogenesis), improved insulin sensitivity in adipose, skeletal muscle, liver, and brain, central (hypothalamic) effects to enhance energy expenditure and leptin sensitivity, and regulation of pancreatic β–cell function to improve glucose tolerance and glucose-stimulated insulin secretion. These findings have led to a proposed mechanism of androgen actions to promote glucose and energy homeostasis via AR-mediated effects on adipose tissue, liver, pancreatic β-cells, skeletal muscle, and metabolic centers in the hypothalamus [48]. Interestingly, aromatization of TES to 17β-estradiol that interacts with the ER appears to be at least partly responsible for preventing abdominal adiposity; thus, both TES and estrogen appear to play important roles in regulation of energy homeostasis in males.

Androgen deprivation therapy (ADT) used in the treatment of prostate cancer, while producing AD, uniquely allows for observation of the direct effects of reductions in TES independent of the influence of other disease processes. Indeed, there is ample evidence of greater incidence of MetS, T2DM, CVD, and cardiovascular death in this patient population [99-101]. The normal aging process in men also results in a gradual but progressive decline in plasma TES. Longitudinal studies reveal declines in plasma TES of 0.4- 2.6% per year with parallel increases in sex hormone binding globulin (SHBG), which then result in a greater decline in free (bioactive) TES of 2% per year. These declines appear to be dependent upon age-related declines in testicular

as well as hypothalamic-pituitary function [102-106]. These progressive declines are associated with gradual changes in metabolic function, leading to greater abdominal adiposity and development of symptoms of MetS and T2DM, including dyslipidemia, hyperglycemia, hypertension, and insulin resistance, and gradual development of CVD [107]. The causality of the relationship between hypogonadism and metabolic disease is not well understood; however, it has been suggested that hypogonadism-induced obesity and obesity-induced hypogonadism likely contribute to a bi-directional effect on the pathogenesis of MetS, T2DM, and CVD because of the disruption in the normal equilibrium between androgen effects on adipose *vs.* skeletal muscle that underpins the normal metabolic phenotype in the male [94,99]. The observations that obesity impairs androgen production while low androgen levels promote increased abdominal fat deposition first led to "hypogonadal-obesity cycle hypothesis" proposed by Cohen in 1999 [108] and later termed the "MetS-hypogonadism relationship [99]. Central to this bi-directional relationship is the role of excess abdominal adipose tissue which functions as an active endocrine organ, beginning with the increased conversion of TES to 17β-estradiol (E2) by the enzyme aromatase expressed in adipose tissue. As well, adipose tissue secretes a number of adipocytokines, which act as circulating endocrine factors, mediating a number of metabolic effects, including inhibition of energy metabolism and insulin sensitivity and increases in inflammatory response and blood coagulation. The elevated circulating levels of E2 exert increased negative feedback on the hypothalamo-pituitary axis (HPA, which normally occurs by local aromatization of TES to E2), reducing luteinizing hormone secretion and subsequent production of TES by the testes. Together with direct inhibitory effects of adipocytokines (pro-inflammatory tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6)) on HPA function (LH secretion) and on testicular TES synthesis, these adipose-mediated derangements result in hypogonadism. The reductions in circulating TES lead to increased deposition of abdominal adipose and further reductions in metabolic rate, thus initiating a vicious cycle, whereby excess abdominal adipose-induced hypogonadism leads to further reductions in metabolism and more

deposition of abdominal adipose tissue and greater hypogonadism (for reviews, see [94,99]. Indeed, the abundance of studies on the hypogonadal-obesity relationship point to the important links observed between hypogonadism, MetS, T2DM, and CVD, as discussed in numerous recent reviews (for reviews, see [94]).

3.2. *Role of androgens in regulation of metabolism in females in health and disease*

The dramatic sexual dimorphism in the metabolic effects of androgens in men *vs.* women is reflected by the striking differences in body fat distribution and skeletal muscle mass observed between the sexes, which begin at birth and are enhanced greatly at puberty with the surge of sex steroid secretion. Thus, men tend to have less total body fat but more abdominal adipose tissue (*i.e.,* android distribution) and greater skeletal muscle mass, driven by the anabolic effects of TES. In contrast, women tend to have more total body fat distributed with a gluteal/femoral and subcutaneous (*i.e.,* gynoid) distribution and less skeletal muscle mass, driven by the metabolic actions of estrogen [49,109,110]. While estrogen in the female mediates the amount, deposition, and function of the metabolically safer gynoid body fat distribution, TES on the other hand drives the deposition and function of the more unfavorable abdominal adipose tissue; however, this is metabolically compensated for by the anabolic effects of TES to increase lean tissue and skeletal muscle mass and function and impair adipogenesis.

The role of TES in the regulation of metabolism in men and the impact of TES deficiency on the development of visceral obesity, insulin resistance, and the MetS is well established; however, the role of androgens in regulation of metabolism in females and the possible impact of abnormalities in androgen levels on metabolic dysfunction in women has not been well studied [111], even though associations between androgen excess and diabetes, obesity, and infertility have been known for nearly a century. Indeed, the relationship between excess androgens and diabetes has been known since the report of “diabetes in bearded women” by Achard and Thiers in 1921 [112]. Similarly, the link between obesity and the triad of polycystic ovaries, hirsuitism,

and oligo/amenorrhea was first reported in 1935 as the Stein-Leventhal Syndrome [113], which was later renamed as polycystic ovary syndrome (PCOS). Thus, similar to the understanding of metabolic effects of TES in men, which has been driven by observations of the metabolic disturbances that occur with abnormalities in androgen levels, so it is with the coexistence of excess androgen levels with cardiovascular risk factors (*i.e.*, dyslipidemia, insulin resistance, obesity) and increased atherosclerosis [114] that occur in PCOS that has advanced the concept that excess androgens exert adverse metabolic effects in women [115,116]. Apart from amenorrhea and infertility, hyperandrogenic conditions in women such as PCOS, congenital adrenal hyperplasia (CAH), and androgenized female-to-male transexuals, are associated with glucose intolerance, insulin resistance, and obesity, and subsequently with T2DM [48]. The observations that the much lower normal levels of TES in females appear to exert beneficial effects on metabolic function, while increases in TES that occur with PCOS or with exogenous androgen therapy in female-to-male transexuals result in cardiovascular and metabolic dysfunction are puzzling and poorly understood [50,117]. Indeed, recent studies utilizing female AR-knockout mice reveal that TES, via the AR, protects against diet-induced atherosclerosis, obesity, and dyslipidemia through modulation of body composition and lipid metabolism [118]. These data suggest that low, normal levels of TES in females exert beneficial metabolic effects on adipose and skeletal muscle tissues. In contrast, with the higher levels of androgens observed in women with PCOS, CAH, or androgenized female-to-male transexuals, metabolic disturbances, including increases in abdominal adiposity, insulin resistance, MetS, and T2DM are observed. These metabolic disturbances are very similar to the metabolic effects of androgen deficiency in men. Thus, female androgen excess and male androgen deficiency are associated with similar adverse metabolic phenotypes, which reveals a dramatic sexual dimorphism in the relationship between TES and metabolism. These observations have led to the concept that a delicate equilibrium exists between androgen effects on adipose tissue *vs.* skeletal muscle that underpins the metabolic phenotype observed with androgen excess in females *vs.* androgen

deficiency in males. This striking sexual dimorphism results in overlapping metabolic disturbances, including abdominal obesity, insulin resistance, the MetS, and T2DM, central risk factors for CVD, and even premature mortality (for reviews, see [49,50]. This concept of overlapping adverse metabolic effects of androgen deficiency in men *vs.* androgen excess in women has been termed the "metabolic valley of death" [50]. (Fig. 5)

The dramatic sexual dimorphism in the relationship between TES and metabolism and the delicate equilibrium of androgen effects in adipose *vs.* skeletal muscle raises the question of what causes the disruption of this equilibrium in androgen effects? Three major factors influencing this balance are gonadal dysfunction, sedentary lifestyle, and normal aging. In the male, these disturbances lead to a deficiency of testicular androgen secretion and the resulting increase in abdominal fat deposition, dyslipidemia, loss of skeletal muscle mass, increased insulin resistance, and development of the MetS and T2DM. In the female, relatively small increases in circulating androgens resulting from PCOS or CAH, and age-dependent reductions in estrogen, in addition to causing phenotypic masculinization, also cause "masculinization" of adipose tissue and its conversion from gluteal/subcutaneous fat to abdominal (visceral) fat deposition with expression of pro-inflammatory cytokines similar to those found in males [119,120]. These findings have led to a proposed mechanism of action involving excess androgens and AR activation, which result in deleterious effects on glucose, fat, and energy homeostasis. These AR-mediated effect include: activation in adipose tissue (increased adiposity and inflammation), central (hypothalamic) effects to reduce energy expenditure and leptin sensitivity, activation of macrophages (oxidative stress), pancreatic β-cell dysfunction (excess insulin), and skeletal muscle (increased insulin resistance), which synergize to promote metabolic dysfunction, inflammation, visceral adiposity, and eventually, T2DM [48].

In summary then, the present data from both animal and human studies reveal the existence of a bi-directional modulation of glucose and fat homeostasis in females *vs*. males. Thus, in males, androgen (or AR) deficiency results in dramatic metabolic dysfunction in aging males but to lesser

extent in females. Since AR activation is weaker in females due to substantially lower circulating androgen levels and a much smaller population of AR in metabolic target tissues, androgens are less important in the maintenance of energy homeostasis in females under normal conditions; however, elevated levels of androgens with PCOS, CAH, or other pathological conditions are sufficient to cause metabolic dysfunction.

4. Summary and conclusions—where do we go from here?

It is increasingly apparent that endogenous TES and other androgens exert widespread beneficial effects on cardiovascular function. Recent experimental animal and human clinical trials over the last 10 years increasingly challenge the long-standing dogma that TES exerts detrimental effects on male cardiovascular health and is largely responsible for the greater incidence of CVD in men than in women. Instead, it is now becoming apparent that it is the gradual decline in circulating TES levels that are a normal part of the aging process that contributes to age-depedent increases in CVD and metabolic dysfunction. Indeed, the incidence of HT and CAD in men increases, while plasma TES level decreases, with age. The intersection of these two plots at middle age suggests (but does not establish causality) that age-dependent increases in HT and CVD are related to the progressive declines in in TES with age (Fig. 6). In parallel, recent experimental animal studies reveal that castration of male rats results in long-term development of HT that is completely reversed by TRT. Further, clinical hypogandism in aging men is associated with both HT and and MetS, which exacerbate the developement of CVD. While the most recent human clinical trials overwhelmingly report that TRT does not increase cardiovascular risk or mortality in older hypogonadal men and that such therapy is associated with reductions in BP and improvement in the MetS (*e.g.,* insulin resistance, serum lipids, diabetes, *etc.*), all of which are risk factors for CVD, clinical trials and observational studies of TRT do not provide unequivocal evidence that exogenous TRT is safe and does not increase the risk of CV events. However, it is also clear that at least some of the human studies suffer from poor experimental design and statistical analysis and investigator bias. Thus, unequivocal proof that TRT is safe and an

efficacious treatment for hypogonadism and associated cardiovascular and metabolic dysfunctions will require more better designed clinical trials that avoid the shortcomings of previous studies. Nevertheless, the growing recognition of the roles of androgens and aging in the pathogenesis of HT are an emerging and important mechanism. Our understanding of the role of androgens in female health as well as CVD and metabolic dysfunction is still relatively limited and future studies are needed to increase the awareness of the mechanisms underlying the striking sexual dimorphism in the cardiovascular and metabolic effects of TES.

An important part of validating the value of TRT in the treatment of CVD and metabolic dysfunction is advancing the study of selective androgen receptor modulators (SARMs), which could provide for selective androgen treatment of cardiovascular or metabolic dysfunction, while avoiding detrimental effects of more conventional androgen analogs, such as the anabolic steroids. Another related topic of great importance is the study of novel endogenous metabolites of TES and other androgens. In particular, the TES metabolite 5β-DHT, which is the genomically inactive stereoisomer of the most potent androgen 5α-DHT, which mediates many of the reproductive effects of TES. While genomicallty inactive as an androgen, 5β-DHT is highly efficacious and potent as a vasodilator under both acute and chronic settings [41,44]. Recent preliminary studies suggest that chronic treatment of castrated male rats with 5β-DHT exerts renal as well as vascular effects that completely reverse castration-induced hypertension after only one week of treatment, whereas treatment with TES requires 5 weeks [121]. Whether 5β-DHT also exerts non-genomic metabolic effects is uncertain, but clearly additional study of this metabolite may be fruitful in the treatment of CVD and metabolic dysfunction related to hypogonadism in men.

Finally, let us address the conundrum of beneficial *vs.* deleterious effects of TES and thereby answer the question: is TES a Janus-faced molecule? Based on the accumulating recent evidence provided in this review, the long-held view that TES is detrimental to male health should

likely be discarded in favor of the view that, at least in men, TES overall is beneficial to cardiovascular and metabolic health and that the apparent conundrum of androgen effects is the result of earlier dogmatic views combined with a limited number of earlier studies that suggested beneficial effects. With the rapidly accumulating beneficial effects of TES reported in the last 10-15 years, it must be concluded that in men, TES is most definitely not a Janus-faced molecule, but rather one that looks only in one direction, that of promoting health. With regard to the role of androgens in women, the situation is quite different and at present, it would appear that TES is a Janus-faced molecule that mediates detrimental as well as beneficial effects in women.

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Table 1. Acute and chronic androgen-induced hypotensive and antihypertensive effects in experimental rat studies *in vivo.*

ANDROGENS	RESPONSES PRODUCED	REFERENCES
5α-, 5β-DHT and its 3α,5β-reduced metabolite	Antagonism of vasopressor responses to noradrenaline or. Bay K 8644 in vagosympath-ectomized pithed rats (**acute**)	Perusquía and Villalón 2002
TES, 5β-DHT	BP reduction (hypotension) in normotensive SD and Tfm rats (**acute**)	Perusquía *et al.,* 2015
TES, 5α- and 5β-DHT	Antihypertensive responses in SHR and CsX-WKY rats (**acute**)	Perusquía *et al.,* 2017
DHEA, TES, 5α- and 5β-DHT	Antihypertensive responses in gestational hypertension (WKY model of preeclampsia) (**acute)**	Perusquía *et al.,* 2018
TES, 5α-DHT	Antihypertensive effects in hypertensive androgen-deficient CsX SD rats (**chronic**)	Hanson *et al*, 2020

DHEA (dehydroepiandrosterone), TES (testosterone), 5α-DHT (5α-dihydrotestosterone), 5β-DHT (5β-dihydrotestosterone), 3α,5β (etiocholanolone); BP (blood pressure), Tfm (testicular-feminized male rat), SHR (spontaneously hypertensive rat), WKY (Wistar-Kyoto rat), SD (Sprague-Dawley rat), CsX (castrated male rats), Bay K 8644 (voltage operated calcium channel agonist).

Figure Legends

Fig. 1. Classical biochemical pathways for synthesis of C19 Androstanes (Testosterone, TES) in males by the Leydig Cells in the testes. TES biosynthesis may proceed by either the Δ4 pathway (via androstenedione, green pathway) or Δ5 pathway (via DHEA and androstenediol, red pathway), intermediates are not shown. Once secreted, circulating TES is subsequently metabolized in peripheral target tissues to the highly potent tissue androgen 5α-dihydrotestosterone (5α-DHT) or to the genomically inactive isomer 5β-DHT. TES is also metabolized in some peripheral tissues, especially adipose, to the C18 Estranes (17β-Estradiol or Estrone). These pathways are also active in females in the ovarian follicle: thecal cells synthesize TES which is then aromatized by neighboring Granulosa cells to 17β-Estradiol.

Enzymes involved: 1) 3β-HSD, 3β-hydroxysteroid dehydrogenase/D5-isomerase;

2) Aromatase; 3) 17β-HSD, 17β-hydroxysteroid dehydrogenase; 4) 5α-reductase;

5) 5β-reductase. Redrawn from Perusquia, 2022 [122].

Fig 2. Pathways and time-courses of classical genomic *vs.* rapid non-genomic mechanisms of androgen actions on target cells. Whereas genomic actions involve entry into the cell and nucleus to alter genomic transcription, translation, and protein synthesis in a longer time frame (hours to days, yellow pathway), more rapid non-genomic actions may involve binding to membrane-associated receptors and activation of G proteins and intracellular signaling cascades, or direct interaction with the cell membrane and modulation of associated ion channels in a much shorter time frame (seconds to minutes, green pathways). Redrawn from Perusquia, 2022 [122].

Fig. 3. Drawing of the Roman God Janus, who was the patron of doorways, transitions, beginnings and endings, and was depicted as having two faces, symbolic of his ability to simultaneously look into the future and the past. The two faces of Janus frequently symbolized transitions such as the past to the future, from one condition to another, and one vision to another. Thus, the two faces of Janus are symbolic of the beneficial *vs.* deleterious effects of androgens on cardiovascular and metabolic functions.Reprinted with permission of the artist, Andrey Kokorin.

Fig. 4. Peripheral metabolism of testosterone to either 5α-dihydrotestosterone (5α-DHT) or 5β-DHT ion androgen target tissues. Note the distinct folding of the A-ring in the 5β-DHT molecule that appears responsible for the loss of androgenic activity in this molecule, but which also confers it with high efficacy and potency as a vasodilator and likely reduces its membrane permeability compared to testosterone).

Fig. 5. Effects of gonadal dysfunction on metabolic function, abdominal obesity, and muscle mass in men *vs.* women. The Y axis represents the percentage of men or women in the general population, the X axis represents serum levels of total TES. TES levels in normal men are 10-30 fold higher than in normal women (note blue *vs.* pink arrows on X-axis). Androgen excess in women (*e.g.,* development of PCOS) increases abdominal adiposity, resulting in an unfavorable metabolic profile (MetS); whereas in men, much higher normal androgen levels protect against MetS by increasing lean and skeletal muscle mass and reducing abdominal adiposity to levels equivalent to those of normal women. The loss of androgen in men results in a marked decline in lean and skeletal muscle mass and increased abdominal adiposity, also resulting in MetS. The grey area in the center represents the common ground of metabolic dysfunction and development of MetS and T2DM, major risk factors for development of CVD in **both** females and males, hence it has been described as the "metabolic valley of death" by Schiffer *et al* [50]. Redrawn from Morreale-Escobar *et al.,* 2014 [49].

Fig 6. Incidence of coronary artery disease (CAD) mortality in white men (blue line) and serum total TES in white men (red line) *vs.* age at each decade. The point of intersection of these two plots at middle age (55-64 year age interval) suggests (but does not establish causality) that the marked increase in CAD mortality after 55 years is related to the marked decline in serum TES prior to this age. Further, clinical hypogonadism in aging men is strongly associated with HT and MetS, central risk factors for the development of CAD. Thus, it appears that it is the loss of TES that exacerbates CAD and may be detrimental to CVD in men. Data from NHANES [123] and Framingham Heart Studies [124].

Fig. 1

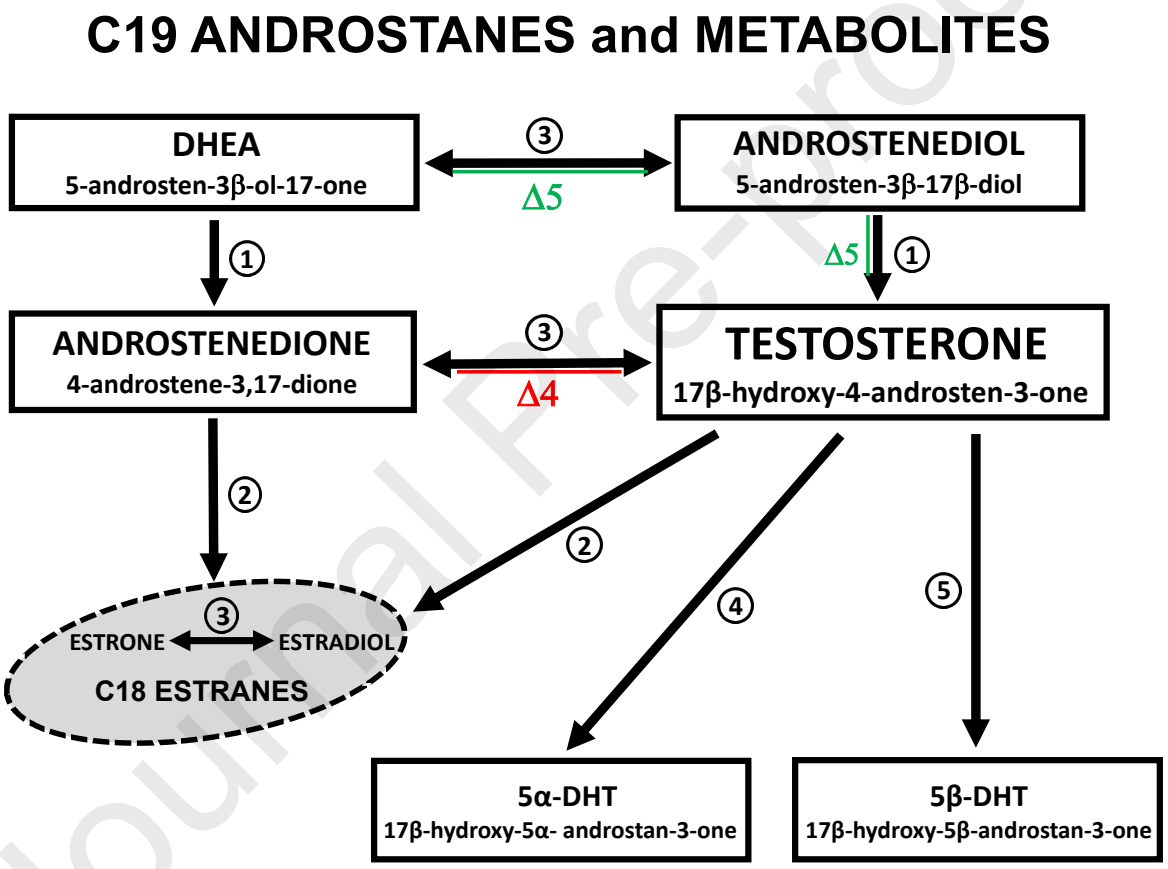


Fig. 2

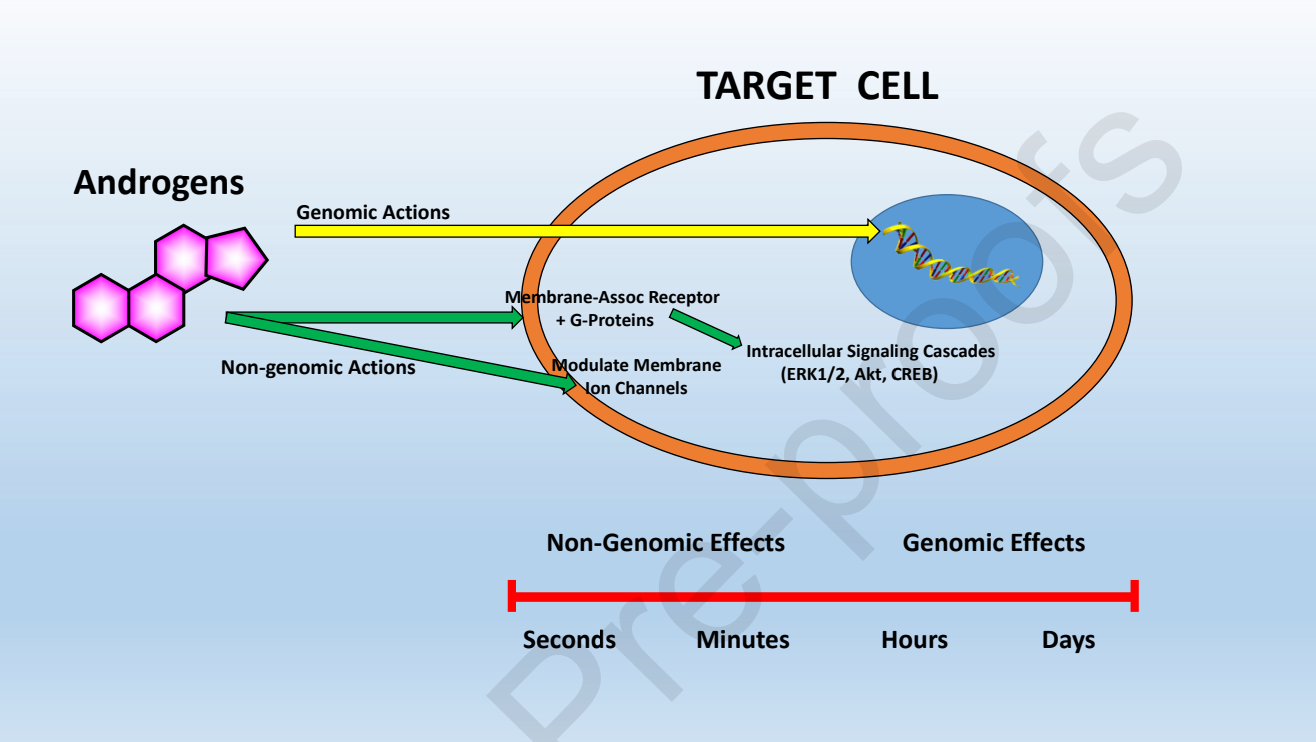


Fig. 3

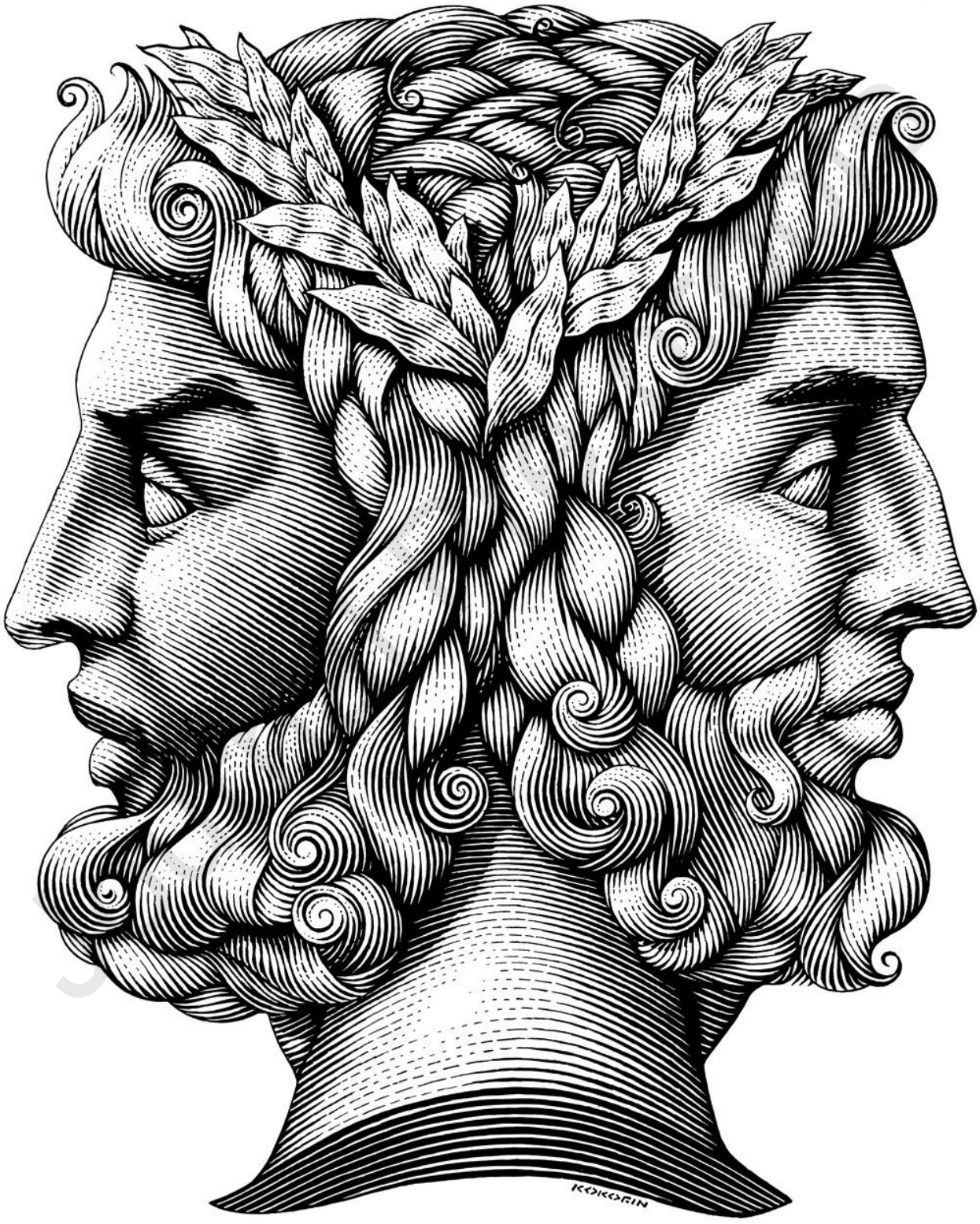


Fig. 4

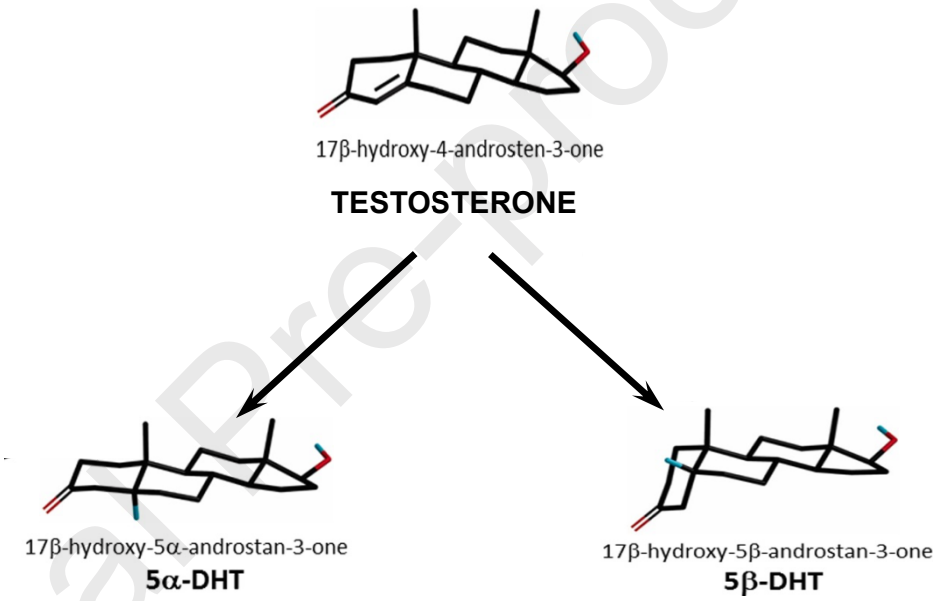


Fig. 5

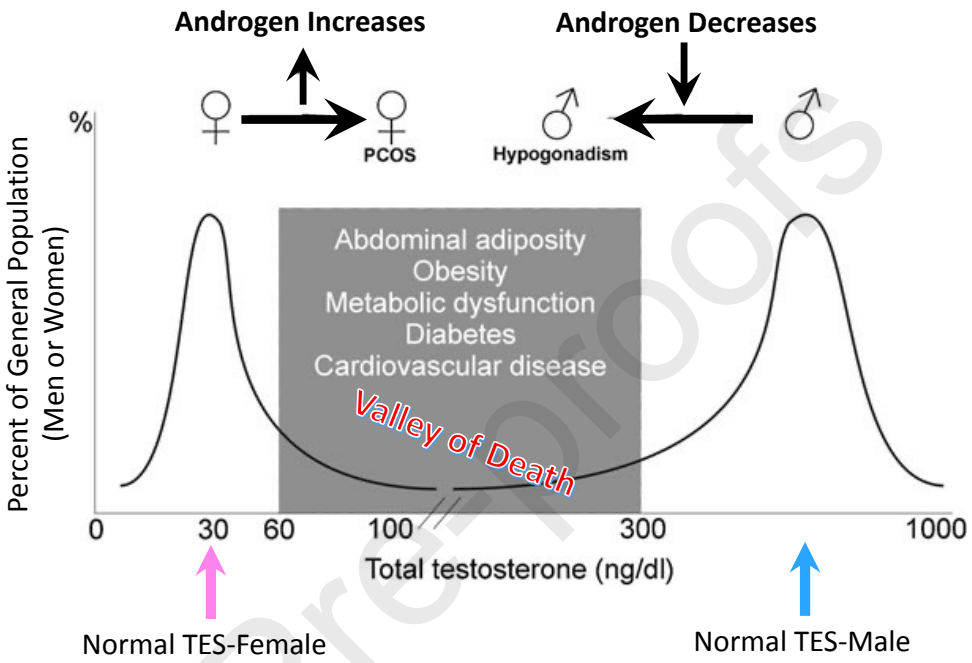
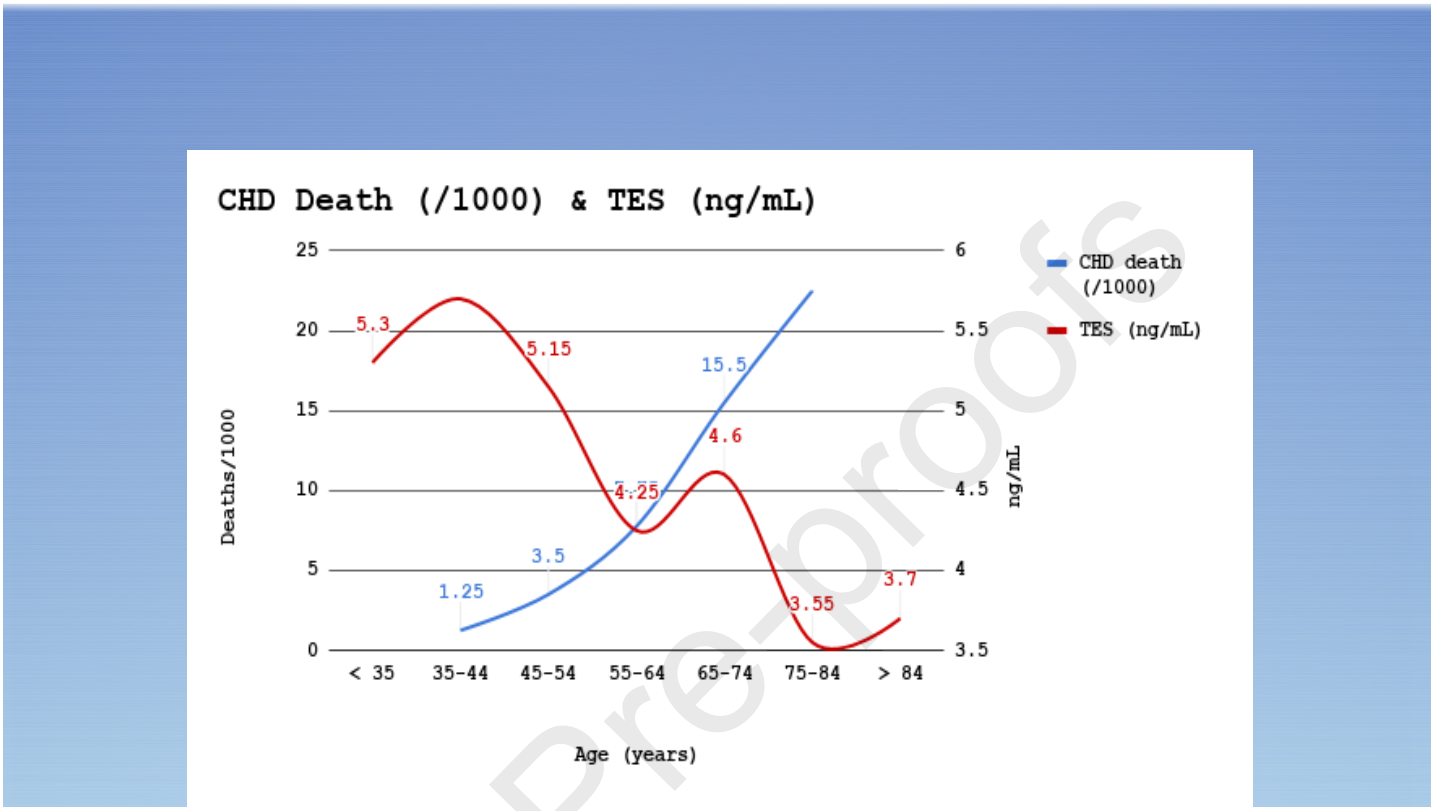


Fig. 6



Increasing levels of testosterone are Janus-faced in exerting beneficial cardiovascular and metabolic effects in males, but deleterious effects in females.

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CREDIT AUTHOR STATEMENT

Drs. Stallone and Oloyo contributed equally to the preparation of this manuscript, which reviewed cardiovascular and metabolic effects of the androgens.