Androgens and Cardiovascular Disease

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Globally, cardiovascular disease will continue causing most human deaths for the foreseeable future. The consistent gender gap in life span of approximately 5.6 yr in all advanced economies must derive from gender differences in age-specific cardiovascular death rates, which rise steeply in parallel for both genders but 5–10 yr earlier in men. The lack of inflection point at modal age of menopause, contrasting with unequivocally estrogen-dependent biological markers like breast cancer or bone density, makes estrogen protection of premenopausal women an unlikely explanation. Limited human data suggest that testosterone exposure does not shorten life span in either gender, and oral estrogen treatment increases risk of cardiovascular death in men as it does in women. Alternatively, androgen exposure in early life (perinatal androgen imprinting) may predispose males to earlier onset of atherosclerosis. Following the recent reevaluation of the estrogen-protection orthodoxy, empirical research has flourished into the role of androgens in the progression of cardiovascular disease, highlighting the need to better understand androgen receptor (AR) coregulators, nongenomic androgen effects, tissue-specific metabolic activation of androgens, and androgen sensitivity. Novel therapeutic targets may arise from understanding how androgens enhance early plaque formation and cause vasodilatation via nongenomic androgen effects on vascular smooth muscle, and how tissue-specific variations in androgen effects are modulated by AR coregulators as well as metabolic activation of testosterone to amplify (via 5α-reductase to form dihydrotestosterone acting on AR) or diversify (via aromatization to estradiol acting upon estrogen receptor αβ) the biological effects of testosterone on the vasculature. Observational studies show that blood testosterone concentrations are consistently lower among men with cardiovascular disease, suggesting a possible preventive role for testosterone therapy, which requires critical evaluation by further prospective studies. Short-term interventional studies show that testosterone produces a modest but consistent improvement in cardiac ischemia over placebo, comparable to the effects of existing antianginal drugs. By contrast, testosterone therapy has no beneficial effects in peripheral arterial disease but has not been evaluated in cerebrovascular disease. Erectile dysfunction is most frequently caused by pelvic arterial insufficiency due to atherosclerosis, and its sentinel relationship to generalized atherosclerosis is insufficiently appreciated. The commonality of risk factor patterns and mechanisms (including endothelial dysfunction) suggests that the efficacy of antiatherogenic therapy is an important challenge with the potential to enhance men’s motivation for prevention and treatment of cardiovascular diseases. (Endocrine Reviews 24: 313–340, 2003)

I. Introduction

Globally, cardiovascular disease will remain the major cause of human deaths well into the 21st century (1). Medical progress in this field has followed identification of pathogenic factors as clues leading to novel mechanistic insights, prevention strategies, and therapeutic targets. This has created specific targets for prevention and/or treatment, including hyperlipidemia, diabetes, smoking, hypertension, clotting mechanisms, and vessel occlusion. Yet, the higher male risk of myocardial ischemia may be the oldest clue to the pathogenesis of atherosclerotic cardiovascular disease. The first known recognizable description of myocardial ischemia, written 2000 yr ago by Seneca (4 BC–65 AD), a Roman stoic philosopher (but not a physician) describing his own disease, recorded that “The attack is very short, and like a storm. It usually ends within an hour . . . . To have any other malady is to be sick, to have this is to be dying” (2). Since then, numerous reports eventually led to systematic clinical observations (2) that have recognized the higher male risk for at least two centuries (3).
Cardiovascular disease shows a consistent male to female ratio of 2.2 (range, 1.2–4.5) among different populations despite wide variations in absolute rates (3).

Over recent decades, the gender disparity in cardiovascular disease has been interpreted primarily as reflecting estrogen-mediated protection against atherosclerosis. Despite remaining unproven by prospective clinical trials, this dominant belief then shaped the direction of much mechanistic research leaving the plausible alternative, i.e., that androgens promote atherosclerosis, little studied. After the first prospective, placebo-controlled, randomized clinical trials (RCT) that showed no cardiovascular benefits of combined estrogen/progestin therapy in menopausal women (4, 5), fundamental rethinking has taken place on whether and how reproductive hormones influence cardiovascular disease, invigorating and reorienting basic and applied biomedical research. This interest is also sharpened by the widening use, misuse, and abuse of androgens in the community. The safety of wider androgen use needs careful scrutiny because even minor deleterious effects on cardiovascular disease, as the most frequent cause of death, are likely to outweigh even substantial perceived benefits from androgens in any medical, lay, or abusive context (6). This review aims to highlight newer findings as well as gaps and opportunities for research relevant to understanding the role of androgens in the male predisposition to earlier onset atherosclerosis as well as the safety of androgens as increasingly used in the community. The role of androgens in lipid metabolism, hemostasis, obesity, and insulin resistance is well reviewed elsewhere (7).

II. Gender, Life Span, and Cardiovascular Disease

A strikingly consistent feature of human populations is the gender gap in life span (8, 9). At birth, life expectancy is consistently shorter for men than women across virtually all populations. In 186 of 191 (97%) United Nations member countries, men have shorter life expectancy by an average of 5.6 yr in those countries above a minimal level [Gross Domestic Product (GDP) in U.S. dollars, $3000 per capita] of economic development (Fig. 1). The gender gap in life span has not narrowed over the last century, and it seems unlikely to diminish without effective targeting. Because cardiovascular disease is the leading cause of human deaths, gender differences in its onset and severity must make an important contribution to the gender gap in life span. This contrasts with causes of death that may be more frequent among men (e.g., trauma, infectious disease) but are quantitatively minor at a population level (10).

Higher male susceptibility to cardiovascular disease may be due to genetic, hormonal, or lifestyle factors or a combination of mechanisms. Genetic contributions due to either Y linkage or X dosage remain largely speculative. The single example is Y chromosome linkage due to the Sry gene, which induces testis formation and the male phenotype including testosterone secretion. This is an interesting circumstance in which a potentially modifiable hormonal pathway is the effector mechanism for a genetic risk, usually considered to be unmodifiable. A gene on the rat Y chromosome responsible for genetic hypertension [spontaneously hypertensive rat (SHR)] involving testosterone, the androgen receptor (AR; Ref. 11), and an unidentified extrarenal (12), endothelium-derived pressor (13) remain to be characterized (14).

There is no evidence for protective dosage effects of any X chromosomal gene, which might hypothetically suppress cardiovascular disease in women, an unlikely mechanism given random X inactivation (lyonization) in females. Gender differences in lifestyle risk factors for cardiovascular disease, notably smoking, diet, physical activity, and other behavioral characteristics, have been the focus of much attention (15) and contribute to, but do not explain, the gender gap in cardiovascular disease (16), but these differences are beyond the scope of this review.

Hormonal effects are the most tractable for practical therapeutics, given the plethora of reproductive steroids available. In recent decades, clinical practice and basic mechanistic studies focused almost exclusively on the estrogen

![Gender Gap in Life Expectancy](https://academic.oup.com/edrv/article-abstract/24/3/313/2424279/314)
protection hypothesis—that cardiovascular disease progression was slowed by estrogenic protection in premenopausal women, a protection lost after menopause. Despite lacking proof by well-controlled prospective studies, the estrogen protection hypothesis became familiar, almost axiomatic. This status was supported by observational case-control studies finding that menopausal women using estrogens had less cardiovascular disease. As established in clinical practice, this dominant hypothesis determined that basic vascular biology studies would focus largely on mechanisms of estrogenic protection from atherosclerosis. In retrospect, selective recruiting of healthier and wealthier women, who are more likely to afford and prefer hormone replacement therapy, into case-control studies gave ultimately false reassurance. This limitation of observational research in eradicating or accounting fully for bias and confounding (17) reemphasizes a division of labor in clinical research, with observational studies being economic and efficient for hypothesis generation but well-controlled interventional studies remaining pivotal, the gold standard, for hypothesis testing. It is salient that, despite decades of clinical practice and supportive observational research, the Heart and Estrogen/Progestin Replacement Study (4) and Women’s Health Initiative (5) studies were the first placebo-controlled RCTs to test the estrogen protection hypothesis.

In fact, the dominance of the estrogen protection hypothesis overlooked contemporaneous evidence (18–20), consistently failing to confirm any break-point in female cardiovascular risk at the expected age of menopause, a key prediction of this hypothesis (21, 22). This contrasts with other definitely estrogen-dependent biological end-points, such as breast cancer and bone density (Fig. 2), that show inflection points in population data at the modal age of menopause. Moreover, cardiovascular death rate curves appear parallel for men and women, apart from an offset of approximately 5 yr in women. This is most consistent with

![Fig. 2. Cardiovascular death rates in women do not exhibit an inflection point at expected modal age of menopause, unlike other unquestionably estrogen-dependent processes such as breast cancer and bone density that show a clear break-point at the expected age of menopause. Cardiovascular death rates in women and men in the United Kingdom, redrawn from the work of Tunstall-Pedoe (21) and plotted on a linear scale (top left panel) or log scale (top right panel), show no inflection point at modal age of menopause and a highly congruent curve displaced (delayed) by approximately 5 yr in women. By contrast, death rates from breast cancer (bottom left panel) and bone density (bottom right panel) show a clear inflection point around the end of the fifth decade of life. Breast cancer and cardiovascular death rates from 1962 U.S. women, redrawn from the work of Tracy (18). Bone density data from the National Health and Nutrition Examination Survey III (1988–94) is based on 6181 white U.S. men and women. [Top panels reproduced with permission from The Lancet (21).]
the same disease process in both genders, but with an early head start in men rather than a continuous exposure to a greater risk in men that would tend to produce diverging lines. Such a head start might reflect biological processes that occur early in life (e.g., perinatal androgen surge in boys) or, perhaps, early in the pathogenesis of atherosclerosis. Furthermore, the estrogen protection hypothesis when applied to men, predicting that estrogen treatment reduces cardiovascular disease deaths, was conclusively refuted by the mid-1970s with two major well-controlled studies showing estrogen treatment of men caused excess cardiovascular deaths. Between 1966 and 1969, the Coronary Drug Project recruited 834 men aged 30–64 yr from 53 centers who were randomized into various treatments or placebo for secondary prevention of myocardial infarction. Both high (5.0 mg) and low (2.5 mg) dose conjugated equine estrogen treatment arms were discontinued prematurely due to excess thromboembolism (23). In the early 1960s, the Veterans Administration Cooperative Urological Research Group recruited 2052 men with prostate cancer from 14 centers (24). Those randomized to treatment with 5.0 mg/d diethylstilbestrol had excess cardiovascular deaths compared with placebo regardless of stage. The excess mortality was a direct estrogen effect, rather than indirectly due to estrogen-induced castration, because the orchidectomy-alone treatment arm did not experience excess mortality. A caveat is that both studies used oral estrogens, which inevitably causes hepatic estrogen overdosage due to first-pass effects, further exaggerated by the high doses used. Because the oral route of administration is a major factor contributing to thrombotic risk among users of estrogen for contraception or menopause, it is tempting to speculate that nonoral delivery of synthetic estrogen partial agonists (“selective estrogen receptor modulators”) might have better outcomes.

Directly testing the hypothesis that adult male testosterone levels accelerate human cardiovascular disease is not feasible. But indirect evidence can be obtained from considering the longevity or mortality of 1) normal men after castration, 2) genetic males who are androgen resistant, and 3) women treated with male androgen doses. Three studies have examined the effects of castration on male longevity. A case-control study of boys who underwent prepubertal orchidectomy (between 1581 and 1858) to preserve singing voice (castrati) showed no difference in life span between 50 castrate (66 ± 2 yr) and 50 intact (64 ± 2 yr) male singers matched for year of birth (25). An independent study confirmed these findings of no difference in life span between 25 castrate (65 ± 2 yr) and 25 intact (65 ± 3 yr) male singers of the same era (between 1605 and 1764; Ref. 26). The privileged social status of opera singers (26) may explain their relatively long lives compared with contemporaries (27). Although limited sample size could theoretically overlook differences (28), the consistent findings and minimal differences in mean ages in both studies make this less likely. Conversely, historical data from Hamilton and Mestler’s 30-yr study (29) of mentally retarded institutionalized men castrated for behavioral control between 1871 and 1932 found that, among white men, median life span of 297 castrated men (69.3 yr) was significantly longer than for 735 intact men (55.7 yr). The survival difference was due to excess mortality from infectious disease among the intact men, but there was no difference in cardiovascular disease or cancer. Castration did not alter life span of nonwhite men or white women. Unfortunately, selective castration to pacify behaviorally difficult inmates (30) introduced a major confounding bias because life expectancy among institutionalized retarded patients is best predicted by independent mobility (31). Chair- or bed-bound patients, susceptible to urinary tract and other infections leading to shortened life expectancy, were less likely to require behavioral control, whereas the more troublesome, mobile patients with normal life expectancy were more likely to undergo castration (30). This selection bias explains the unusually short life expectancy of intact men and the excess deaths from chronic urinary and other infections, whereas the castrate had normal rather than prolonged life expectancy. Overall, there is little sound evidence that androgen exposure shortens men’s lives. The surprisingly limited data of the effects of castration on the life span of domestic cats (32), dogs (33, 34), and rats (35) are inconclusive.

The natural history of complete androgen resistance due to a mutated, nonfunctional AR (36), combining male genetic sex with female phenotype, would provide a decisive test of whether life span or cardiovascular mortality followed male or female patterns. However, standard medical care of this rare disorder abrogates its natural history, making it unlikely that such data will ever be available. Alternatively, female-to-male (F2M) transgender, in which genetic females receive male testosterone doses, provides useful information. The only available follow-up study reported no excess cardiovascular disease among 293 F2M transsexuals during 2418 patient-years of exposure, compared with the general population (37). Longer and larger follow-up studies of such populations would be of considerable interest. Lower but more sustained exposure to androgen excess in women with polycystic ovary syndrome is also associated with no excess of cardiovascular disease (38) or mortality (39), despite increases in cardiovascular risk factors (40, 41), endometrial (but not breast) cancer (42), and more extensive atherosclerosis at coronary angiography (43). Because it is highly unlikely that these studies could have overlooked the greatly increased (7.4-fold) risk predicted by risk factor modeling (44), this striking discrepancy raises the possibility that androgens may have beneficial as well as detrimental effects on atherogenesis in women. If perinatal androgen imprinting is important in triggering male-pattern cardiovascular risk, both F2M transgender and women with polycystic ovary syndrome would lack this exposure, which might explain their relative freedom from cardiovascular complications.

The cardiovascular effects of androgen abuse have been extensively reviewed (7, 45–47). Adverse cardiovascular effects including myocardial infarction, hypertension, arrhythmia, cardiac failure, pulmonary embolism, stroke, and sudden death have been associated with androgen abuse, based on the temporal relationship to usage. Virtually all are single case reports of events that can occur in the absence of androgen usage and, in some, underlying medical disorders contribute to the risks (48). Without knowing the denominator of community exposure, the actual individual risks of such adverse effects compared with the general community
are not clear (49). Given the highly prevalent and sustained usage among elite power athletes and bodybuilders for the last four decades, it is conceivable that the actual risks may not prove high. The East German national sports doping program involved more than 2000 elite athletes annually being treated with high-dose synthetic androgens. Regular medical monitoring recorded no cardiovascular complications, whereas liver disease and many other complications were regularly recorded (50). The longevity of former elite athletes who may have abused androgens may also shed light on the cardiovascular risks of androgen abuse. In a study of 2613 Finnish former elite athletes and age- and locale-matched conscripts, endurance and team athletes had lower cardiovascular mortality and longer lives than the general population (51), consistent with findings among Italian track and field athletes (52) and Dutch distance skaters (53), whereas those in power sports had less extended lives (51). Finnish power lifters among whom androgen abuse was suspected but not verified had 4.6-fold higher overall mortality, although life expectancy and cardiovascular mortality were the same as community controls (54). Further studies are needed to clarify the actual long-term cardiovascular and other risks associated with androgen abuse in former athletes.

In summary, the gender gap in life span is consistent in all but the poorest countries. The best available data suggest that adult male androgen exposure does not shorten men’s life span, but oral estrogen treatment has deleterious cardiovascular effects in men, as it does in women. How much of this harm is due to the oral route of administration or the use of synthetic estrogens is not clear. The risk of harmful cardiovascular effects due to high-dose androgen exposure from androgen abuse needs further clarification. If the gender differences in cardiovascular disease, which must contribute to the gender gap in life span, are due to hormonal effects, they presumably operate early in the pathogenesis of atherosclerosis.

III. Newer Aspects of Androgen Action

Expanding knowledge of androgen physiology has shed light on new mechanisms that may be crucial to better understanding androgen action on the vasculature for both basic and clinical research.

A. Genomic regulation of androgen sensitivity

The classical pathway of androgen action (Fig. 3) involves steroid binding to the AR, a ligand-activated transcription factor, and single copy member of the nuclear receptor superfamily, acting on the genome (36). The genomic action of AR is modulated by a large variety of coregulators, which are proteins that fine-tune target gene expression by enhancing (coactivator) or restraining (corepressor) transcription (55). Although testosterone circulates throughout the body, the factors responsible for variation in tissue androgen sensitivity remain to be further clarified. Intensity of expression of the single human AR (56) partly defines androgen sensitivity, but AR is almost ubiquitously expressed to some degree in tissues. Further biological determinants of tissue androgen sensitivity, including the functional AR polymorphisms as well as tissue distribution and regulation of AR coregulators, androgen metabolic enzymes, and nongenomic mechanisms, remain to be better defined so that their net integrated effects can be understood better.

At present, knowledge of the regulation and tissue distribution of AR coregulators is limited. One study found distinctive tissue distribution of four coregulators in the rat. Furthermore, rat pituitary expression of steroid receptor coactivator-1 showed in vivo hormonal regulation with increases after exogenous T₃ treatment and decreases after exogenous estradiol treatment, the latter corresponding to the markedly lower levels of steroid receptor coactivator-1 mRNA in female compared with male rat pituitaries (57). Another study found highly distinctive expression of 11 coregulators in 6 human breast cancer cell lines, consistent with the suggestion that such complex patterns may modulate distinctive androgen responses of different cell lines and tissues (58). An important new mechanism whereby coregulators can change androgen sensitivity was recently identified in men with recurrent prostate cancer after castration, in whom up-regulation of two AR coactivators potently increases cellular androgen sensitivity (59). Further studies of differential tissue distribution of coregulators are needed to determine whether coregulator distribution or function explains distinctive tissue effects of androgens, including in vascular tissue. There remain many unanswered questions about the modulatory role of AR coregulators in vascular physiology.

Androgen sensitivity could be modulated by a functional polymorphism of AR that influences the strength of the genomic signal transduced from its interaction with an androgen as a bound ligand. One such functional AR polymorphism is the exon 1 triplet repeat CAG (polyglutamine) whereby the repeat length is inversely correlated with androgen sensitivity. Within the normal population, shorter repeat lengths are associated with higher risk of prostate cancer (60), whereas longer repeats are associated with reduced androgen effects on lipids and vascular reactivity (61) as well as bone (62) and sperm production (63) within the normal male population. Pathological extension (>40 repeats) causes a motor neuron disease (Kennedy’s syndrome, spinal muscular bulbar atrophy) with associated androgen insensitivity (64). Whether such fine-tuning of androgen responsiveness influences ultimate cardiovascular outcomes remains to be studied further. Further analysis of this and similar functional polymorphisms of androgen metabolizing enzymes (5α-reductase, aromatase, CYP3A4; Ref. 65) may shed new light on androgen action on the cardiovascular system.

The development of the first nonsteroidal androgens (66, 67) has promise in exploiting tissue-specific differences in androgen sensitivity (68). Whether such tissue-specific partial androgen agonists (selective AR modulators), which are structurally nonaromatizable and functionally dihydrotestosterone (DHT) analogs, would have useful roles in vascular therapeutics mediated via AR remains to be clarified. Pharmacological targeting of nongenomic androgenic vasodilator mechanisms in vascular smooth muscle seems promising, in
contrast to endothelial and vessel wall mechanisms that involve aromatization.

**B. Nongenomic effects**

There is now considerable evidence for rapid, nongenomic effects of steroids (Fig. 3), including androgens (69). Non-genomic steroid action is distinguished from genomic effects by 1) rapid onset (seconds to minutes) that is faster than genomic mechanisms, 2) insensitivity to inhibition of RNA and protein synthesis, 3) effects produced by steroids unable to access the nucleus (either covalently linked to membrane impermeable macromolecules or in cells lacking a nucleus), and 4) not usually blocked by classical antagonists due to different steroidal specificity from classical cognate nuclear receptors. As for other steroids, nongenomic androgen effects characteristically involve the rapid induction of conventional second messenger signal transduction cascades, including increases in cytosolic calcium and activation of protein kinase A, protein kinase C, and MAPK, leading to diverse cellular effects including smooth muscle relaxation, neuromuscular and junctional signal transmission and neuronal plasticity (70). In addition, nontranscriptional mechanisms have also been reported (71). Most nongenomic effects involve a membrane receptor, and putative binding sites are described for all major classes of steroids (72), including androgens (70). In mammals, only a membrane receptor for progesterone has been cloned (73, 74), but functional characterization is lacking. A membrane progestin receptor cloned from fish ovary features a heptahelical transmembrane structure typical of G protein-coupled receptors, and the recombinant protein exhibits high-affinity binding of progesterone with activation of postreceptor signal transduction pathways (75). No membrane AR has been characterized, but preliminary evidence of a low-affinity microsomal membrane binding site for alkylated androgens (76) and an endothelial cell plasma membrane dehydroepiandrosterone (DHEA) binding site (77) still require functional proof of specific receptor status. A plasma membrane SHBG re-

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**Fig. 3.** Nongenomic and genomic androgen mechanisms. **Left,** Nongenomic effects of testosterone, which are triggered by binding to a still uncharacterized (nonclassical) membrane receptor. This activates second messengers including calcium and protein kinases, which produce typically rapid responses. **Genomic effects are depicted in the middle and on the right.** Testosterone (or other aromatizable synthetic androgens) crosses the cell membrane to be converted to estradiol (or the aromatic synthetic analog) by aromatase, which then binds to and activates ERs (the right, DHT) or other nonaromatizable androgens (or ERs). Right, DHT (or other nonaromatizable androgens) enters the cell to bind and activate the AR. Ligand-bound ER or AR dissociate their heat-shock protein (HSP), undergo conformational changes, dimerize, and translocate into the nucleus where they bind to specific sites known as estrogen response elements (ERE) or androgen response elements (ARE) located within the DNA of target nuclear genes to produce long-term genomic effects of testosterone.
ceptor capable of modulating androgen action at plasma membranes and initiating intracellular cAMP signaling has been described in humans (78). The SHBG receptor remains to be fully characterized, and it is not clear whether it has any physiological role in species like rodents that lack circulating SHBG.

C. Metabolic activation of testosterone

A key issue in the biological effects of testosterone is its conversion to bioactive metabolites (Fig. 4). Although only a small fraction (<5%) of testosterone output undergoes such transformation usually in local tissues, conversion both amplifies and diversifies testosterone action. Conversion to its 5α-reduced metabolite, DHT, by either type 1 or type 2 5α-reductase amplifies testosterone action because DHT has higher molar potency due to its more avid binding affinity and slower dissociation rate from the AR (79). In the prostate, feed-forward induction by DHT of type 2 5α-reductase (80) results in virtually all testosterone entering the prostate being converted to DHT, thereby greatly enhancing local AR-mediated effects. Type 1 and type 2 5α-reductase has been identified in vascular tissues based on immunoreactivity (81, 82) and enzymatic activity (83–85), but the biological consequences of androgen amplification in vessel walls remain to be clarified (84, 86, 87). Furthermore, conversion of testosterone to estradiol by the enzyme aromatase (CYP19) diversifies androgen action by activating estrogen receptors (ER). Aromatase gene expression (88–91), protein (90–92), and enzymatic activity (93) have been detected in vascular tissues, including human coronary arteries (92) particularly in endothelium and smooth muscle. A more complete picture of gender differences and sex hormone regulation of aromatase and 5α-reductase activity in vascular tissues is needed.

D. Derived measures of testosterone and SHBG

In clinical studies and physiological research on androgen action in vivo, including on cardiovascular disease (94), there is increasing reference to derived testosterone measures, using names such as free, bioavailable, or free androgen (or testosterone) index (95, 96). Beyond the standard immunoassay measurements of blood testosterone, these derived measures require additional assays and/or calculations based on the assumption that they measure a more biologically active fraction of blood testosterone. This approach reflects the free hormone (or free hormone transport) hy-

**Fig. 4.** Pathways of testosterone action. In men, most (>95%) testosterone is produced under LH stimulation through its specific receptor, a heptahelical G protein-coupled receptor located on the surface membrane of the steroidogenic Leydig cells. The daily production of testosterone (5–7 mg) is disposed along one of four major pathways. The direct pathway of testosterone action is characteristic of skeletal muscle in which testosterone itself binds to and activates the AR. In such tissues there is little metabolism of testosterone to biologically active metabolites. The amplification pathway is characteristic of the prostate and hair follicle in which testosterone is converted by the type 2 5α-reductase enzyme into the more potent androgen, DHT. This pathway produces local tissue-based enhancement of androgen action in specific tissues according to where this pathway is operative. The local amplification mechanism was the basis for development of prostate-selective inhibitors of androgen action via 5α-reductase inhibition, the forerunner being finasteride. The diversification pathway of testosterone action allows testosterone to modulate its biological effects via estrogenic effects that often differ from AR-mediated effects. The diversification pathway, characteristic of bone and brain, involves the conversion of testosterone to estradiol by the enzyme aromatase, which then interacts with ERα and/or ERβ. Finally, the inactivation pathway occurs mainly in the liver, with oxidation and conjugation to biologically inactive metabolites that are excreted by the liver into the bile and by the kidney into the urine.
hypothesis based on theoretical considerations by Ekins (97, 98), Pardridge (99–101), and Mendel (102) but remains contentious and unproven (103). The physiological background to this hypothesis is that nonpolar steroid hormones, having very low solubility in aqueous extracellular fluid, circulate in the bloodstream largely bound to specific high-affinity, low-capacity circulating transport/carrier proteins (e.g., SHBG, corticosteroid-binding protein, T4-binding globulin), as well as binding to lower affinity (but high capacity) nonspecific binding proteins (e.g., albumin) with only a very small fraction (~1–2%) not bound to any circulating protein (104). Tracer experiments confirm that nonprotein-bound steroid is transported most rapidly from bloodstream to tissues (101). The free hormone hypothesis states that the nonprotein bound fraction is the most biologically active moiety of a circulating steroid hormone with the protein-bound moiety a reserve, biologically inactive buffer. Despite widespread but uncritical adoption, this sophisticated-sounding concept lacks theoretical or empirical validity. In theoretical terms, if nonprotein-bound steroid is transported more rapidly to target tissues (representing a pathway of accelerated bioactivity) as well as to hepatic sites of steroid degradation (representing termination of hormone action). The net balance between these two effects is inherently unpredictable, depending on dynamic balance between many factors, including the relative mass and blood flow of target and metabolic tissues. Consequently, there is no theoretical basis to believe that free hormone measurements necessarily represent a more biologically active (rather than more rapidly inactivated) moiety of a circulating steroid. The demonstration that SHBG-bound testosterone is biologically active, via binding to cell surface SHBG receptor (105), further undermines the free hormone hypothesis, which predicts that testosterone tightly bound to SHBG would form a biologically inactive buffer reservoir. These studies do highlight that SHBG, usually considered a complex indicator of net steroid action on its hepatic synthesis and secretion (106), may have a biological role (78), but evidence of cardiovascular effects remains speculative (94, 107–109). Finally, the absence of a circulating SHBG in rodents (110) removes any basis for the free hormone hypothesis in physiological research on androgen action involving rodents.

Despite lacking theoretical validity, such derived testosterone measures might still be useful empirically if they provided prediction of, or correlation with, independent biological androgen effects that were superior to those of standard testosterone measurements. Such empirical validation, however, is conspicuously lacking. Some derived testosterone measures clearly lack face validity. For example, among derived measures purporting to estimate free testosterone, both the free androgen (or testosterone) index calculation (111) and the free testosterone analog assays (112–114) are well-known to be invalid in men. Other measures such as equilibrium dialysis to estimate the free fraction or the bioavailable (corresponding to free plus loosely albumin-bound fractions) may be technically reproducible (95, 96), but there is no evidence that they provide significantly superior or additional biological information to the measurement of total testosterone as judged by independent biological effects. In summary, derived testosterone measures lack theoretical or empirical validity, add little or no explanatory power to clinical or physiological research beyond measurement of total testosterone, and should be used, if at all, only with concomitant independent empirical validation of those measures. Further studies of the biological role of SHBG in modulating androgen action are needed.

E. Fetal programming and perinatal androgen imprinting

The role of early life environmental exposures on late-life cardiovascular disease has recently been recognized. Barker (115, 116) has compiled evidence from a wide variety of sources implicating prenatal environmental programming as a major determinant of susceptibility to diseases of later life, notably cardiovascular disease (115) and type 2 diabetes (116), the latter termed the thrifty phenotype hypothesis. He proposes that fetal adaptation to intrauterine malnutrition, to prioritize protection of vital organs, conditions preferred metabolic pathways in developing organ systems (developmental plasticity). The perpetuation of such adaptations into postnatal life may foster mechanisms that are deleterious in adult life. The precise mechanisms leading to cardiovascular disease remain to be elucidated, but accelerated early growth following low birth weight is characteristic (117), especially in boys (118). Neonatal androgen imprinting determines the sexually dimorphic mature blood pressure patterns of SHR rats (119) and susceptibility to diet-induced hypertension (119). This may be an important clue, when considered in conjunction with the natural history of gender differences in cardiovascular mortality, that points to atherogenesis having similar progression in men and women but with men having a head start at some undefined early stage in pathogenesis. A key event in early male life is the perinatal androgen surge when blood testosterone concentrations reach adult levels for months. This epoch is critical for hormonal imprinting of brain (120), prostate (121–123), and probably other androgen-sensitive tissues, perhaps including vascular tissues. An informative clinical model to test the effects of perinatal androgen exposure is congenital adrenal hyperplasia. Women with 21-hydroxylase deficiency would usually experience marked androgen excess until effective treatment is instituted. This early life androgen excess is known to influence gender identity and role (124), but effects on life span and cardiovascular disease are not known. Interestingly, castration of inbred rats at birth prolongs life span, whereas castration at weaning or maturity has no effect (35). More specific examination of gender in relation to fetal environmental programming and related perinatal events, such as the androgen surge and hormonal imprinting, may be informative.

IV. Vascular Biology

A. Gender

Gender differences are characteristic of animal models of atherosclerosis. Males develop earlier and more extensive atherosclerotic plaques independent of lipid levels in diet-induced models in the nonhuman primate (125) and in rabbits with (126) and without (127) intimal injury. Gender
B. Androgen treatment

Testosterone treatment consistently inhibits atherosclerosis in castrate, cholesterol-fed male rabbits (141–143). The most detailed study showed that castration of male rabbits increased, whereas both testosterone and DHEA treatment inhibited, aortic atherosclerosis. The higher testosterone concentrations produced by an injectable testosterone ester provided significantly better protection than an oral testosterone ester or DHEA. Testosterone effects were not explained by lipid mechanisms, whereas aortic ER (but not AR) content was down-regulated in parallel with atheroprotective effects. This suggestion of the importance of aromatization within the vessel wall for atheroprotection has been supported by observations that treatment with DHEA (readily aromatized) inhibits atherosclerosis in intact, cholesterol-fed rabbits (144–146), whereas a synthetic nonaromatizable androgen (stanozolol) had no protective effect (147). Similarly, in LDL receptor-deficient mice, an aromatase inhibitor (anastrozole) blocked the atheroprotective effect of both endogenous and exogenous testosterone (91). In female animals, however, proatherogenic effects of androgens are reported. Treatment of ovariectomized nonhuman primates for 1–2 yr with nandrolone decanoate (148) or 8 months with testosterone (149) increased coronary artery atherosclerotic plaque size compared with treatment controls and lower level androgen exposure (androstenedione plus estrone). The enhancement of atherogenesis was not explained by lipid changes or by the limited aromatizability of nandrolone. However, androgens also enlarged coronary diameter (148) and enhanced endothelium-dependent acetylcholine vasodilator responses (149), consistent with vasodilator effects of androgens.

In ApoE-deficient male mice, atherosclerotic plaque size is decreased by estradiol treatment of intact (150) or orchidectomized (151, 152) males. However, reported effects of testosterone are conflicting. A study using a GnRH antagonist (Cetrorelix) to deplete endogenous testosterone found that castration reduces, and testosterone treatment increases, atherosclerosis (133), whereas another study reported that orchidectomy had no effect and testosterone treatment reduces atherosclerosis (152). In LDL receptor-deficient male mice, orchidectomy increases, whereas testosterone or estradiol treatment reduces, atherosclerosis, and an aromatase inhibitor (anastrozole) blocks the atheroprotective effects of endogenous and exogenous testosterone (91). The speculation that these differences are due to extragonadal LH effects (133) lacks basis because no vascular effects of LH outside the reproductive tract have been established (153). Overall, animal models do not yet provide a fully coherent picture, but most evidence supports testosterone having an atheroprotective effect requiring aromatization in males, whereas in females androgens are proatherogenic. The role of 5α-reduction in these testosterone effects has not been reported. Further studies using pharmacological probes (nonaromatizable androgens, blockers of androgen metabolism) and genetic mouse models to dissect components of testosterone action on the vasculature will be of continuing interest.

C. Genomic effects

AR is expressed in all cells of the vasculature, including endothelial cells, smooth muscle cells, myocardial fibers, macrophages, and platelets (Fig. 5). In earlier studies, myocardial (154) and aortic (155–159) AR content was similar in male and female rats (155, 156, 159), rabbits (158), and non-human primates (154, 157). However, males showed more nuclear localization, consistent with greater AR activation by endogenous testosterone (154–157). Recent studies using more sensitive detection methods find consistent gender differences in vascular tissue AR content. Higher AR expression in males is reported for rat vascular smooth muscle (160), human macrophages from peripheral blood (161) or synovium (162), and mesenteric artery and endothelial cells (163). Hormonal regulation of AR protein levels in nonreproductive tissues including the vasculature is not well defined. Ligand binding initially stabilizes AR protein, but prolonged exposure leads to down-regulation (164). Male-specific expression of AR, such as in macrophages and smooth muscle cells, implies long-term AR protein up-regulation with prolonged exposure to endogenous male testosterone concentrations. Short-term exposure of rabbit arterial neointimal plaque to testosterone increased AR mRNA while inhibiting plaque development in culture (165).

Atherosclerosis involves interaction between the cells of the arterial wall (endothelial and smooth muscle cells) with those migrating into it (macrophages) (166). Monocyte adherence to the vascular endothelium, among the earliest detectable abnormalities in arteries of hypercholesterolemic animals, is followed by migration into the artery wall to form foam cells (fatty streak) (167). Gender differences along this pathogenic pathway have been described. Monocyte binding to aortic endothelial cells is higher in male than female hypercholesterolemic rabbits, but binding was lower (equal in male and female rabbits) if blood cholesterol levels were not elevated (126). Such monocyte binding to the endothelium requires cell adhesion molecules with gender-specific expression, a prediction fulfilled by the identification of vasa-
cular cell adhesion molecule-1 (VCAM-1) involvement (168). Cultured human umbilical vein endothelial cells (HUVEC) from a male donor demonstrate increased expression of VCAM-1 gene and cell surface protein levels after stimulation with DHT, a nonaromatizable androgen. The DHT effect is dose-dependent, blocked by flutamide and expressed in a gender-specific manner involving a nuclear factor-kB mechanism (168, 169). Furthermore, an antibody to VCAM-1 blocks the enhanced monocyte adhesion induced by DHT (168). These findings were confirmed in HUVEC (170), whereas testosterone inhibits TNF-α stimulated VCAM-1 expression in human aortic endothelial cells (171) and in HUVEC from a female donor (172). In the latter study, the lack of DHT effect is expected for cells of female origin because they lack AR expression (161). By contrast, estradiol inhibits VCAM-1 mRNA and protein expression so that blockade of the effects of testosterone on VCAM-1 by an aromatase inhibitor (anastrozole) or an ER antagonist (ICI-182780) reflects inhibitory estradiol effect unopposed by AR-mediated effects in female HUVEC, which lack AR expression. Ultimately, AR-mediated androgenic stimulation of VCAM-1 in endothelial cells coupled with the male selective AR expression (161) suggests that the earliest steps of atherogenesis are markedly different between genders. Whether this represents the early-stage head start that determines the male predisposition to cardiovascular mortality in later life remains to be determined.

Apoptotic damage of vascular endothelial cells is an important cellular mechanism in atherogenesis, leading to increased platelet adhesiveness and thus increased tendency for thrombus formation (173). Testosterone enhances endothelial cell apoptosis provoked by serum deprivation (as an in vitro model of arterial wall damage; Ref. 174). This testosterone effect is blocked by flutamide but is not replicated by DHEA or estradiol, indicating involvement of AR but not aromatization. Any role of 5α-reduction has not been reported.

In the intact arterial wall, smooth muscle cells regulate the arterial tone and produce the extracellular matrix. Proliferation and migration of smooth muscle cells are important steps in the formation of neointima and stenoses. Testosterone and DHT treatment stimulate proliferation of rat vascular smooth muscle cells (84), whereas estradiol inhibits their proliferation and migration (175, 176). Consequently, postinjury neointimal plaque size is larger in males than females and increases after ovariectomy (but not orchidectomy) with restoration of intact female levels by estradiol replacement (177).

Macrophages play a key role in atherosclerosis by migrating into the vessel wall where they internalize large amounts of exogenous lipids by various unregulated scavenger receptors and phagocytosis. Lipid accumulation transforms macrophages into foam cells, forming the fatty streaks characteristic of early atherosclerosis. Experimentally, androgens
enhance foam cell formation because lipid loading of male (but not female) macrophages is increased by DHT treatment, an effect blocked by flutamide consistent with an AR-dependent mechanism (161). Conversely, testosterone also enhances reverse cholesterol transport, which might retard development of fatty streaks. Testosterone at a physiological concentration increases expression of the scavenger receptor-B1 at mRNA and protein levels in human hepatocytes and monocyte-derived macrophages (178). The functional consequences of increased scavenger receptor-B1 were observed as testosterone-dependent enhanced transfer of cholesterol esters from monocyte-derived macrophages to high-density lipoprotein-3 during in vitro culture (178). Whether the testosterone effect required the AR or aromatization was not reported, but because estrogens reduce scavenger receptor-B1 in nonsteroidogenic tissues (179), aromatization is not required. Any role of 5α-reduction has not been reported. Testosterone treatment of murine macrophages inhibits nitrite release via inhibition of the inducible nitric oxide (NO) synthase (NOS) enzyme, although the underlying mechanism remains unknown (180). This inhibition of inducible NOS could increase platelet aggregation and thrombosis risk associated with androgen treatment by eliminating the antiaggregatory effects of NO.

D. Nongenomic effects

Among vascular cells, nongenomic effects of testosterone (Fig. 5) are described for macrophages (181, 182), endothelial cells (163, 183), and vascular smooth muscle. Macrophage cell lines (IC-21, RAW264.7) that do not express the nuclear AR show rapid and repeated increased cytosolic calcium responses to testosterone, DHT, and testosterone rendered membrane-impermeable by conjugation to BSA (T-BSA) but not to inactive androgens (5β-DHT, 1-DHT) (181, 182). Testosterone effects were not blocked by classical androgens (cyproterone, flutamide) or antiestrogens (raloxifene, tamoxifen, ICI182, 780) excluding the involvement of AR and aromatization, but the role of 5α-reduction remains unclear. T-BSA was bound to plasma membranes and internalized by an energy- and cytoskeleton-dependent process. Based on blocker experiments, the calcium response originated from primarily internal stores coupled to phospholipase C via a G protein-coupled receptor (184). In RAW264.7, murine macrophages stably transfected with c-fos promoter (RAW-fos13), the functional effects of nongenomic testosterone signaling were illustrated by testosterone attenuation of lipopolysaccharide-activated c-fos promoter activity, p38MAPK, and NO production (182). In human endothelial cells, testosterone, T-BSA, and DHT all stimulate increased cytosolic calcium via membrane influx that was abolished by removing extracellular calcium or blocking membrane calcium channels but not by blockade of intracellular calcium stores (163). By contrast, in cultured rat, aortic endothelial cells testosterone inhibited bradykinin-induced increases in intracellular calcium but had no effect itself (183).

Testosterone-induced vasodilatation, first reported by Waldman in 1945 (185), is well established with in vitro vasodilator effects reported in precontracted arteries of the rat (13, 186–190), mice (191), rabbit (192), pig (193, 194), guinea pig (195), ferret (196), and dog (197). These effects involve primarily the vascular smooth muscle without requiring the presence of endothelium, although an endothelial contribution is apparent in some studies (186, 197). Testosterone acts via a nongenomic mechanism because the responses are rapid; present in ftns with mutated, non-functional AR (186, 191, 198); reproduced by testosterone conjugated to membrane-impermeable macromolecules (189, 199, 200); and not blocked by inhibitors of DNA and protein synthesis (199, 201), classical AR antagonists (190, 199, 201, 202), aromatase inhibition (190, 192, 203), or ER blockers (197, 203). The mechanisms involved include endothelium-derived NO (where endothelium is involved) and, more regularly, blockade of membrane calcium influx via voltage-operated calcium channels (191, 204) and potassium efflux involving voltage-operated (13, 189) and BKCa (194) potassium channels (191) in vascular smooth muscle, including in humans (205). The steroideal selectivity of the putative membrane receptor involved in testosterone-induced vasodilation is unusual, with classical antiandrogens (cyproterone, flutamide) ineffective, whereas 5β-androstanes (androsterone, etiocholanolone, 5β-DHT) are more potent vasodilators than 3α-androstanes (5α-DHT, 3α-diol), which is the reverse of their potency at the AR. Neither 3 nor 17 conjugation impairs vasodilatory activity (200). Testosterone induces vasodilatation in all arterial beds studied, comprising coronary, mesenteric, iliac, renal, and femoral (206), although sensitivity varies between them (192, 197, 206–208) and is reduced by aging (209).

The consistent requirement for high (micromolar) concentrations of testosterone for vasodilatory responses raises questions about the physiological nature of these responses. Few studies are reported at lower testosterone doses, but a recent in vivo study of pigs reported widespread arterial vasodilatatory responses using testosterone infusions calculated to produce concentrations of approximately 1 µg/liter (206). At similar physiological concentrations of testosterone, however, vasoconstrictor responses have been observed (199, 201), including one study in which the higher, supra-physiological testosterone concentrations produced vasodilation (201). Vasoconstrictor responses have long been known in tissues obtained from androgen-treated animals (195, 210–213), but the effective tissue androgen concentrations involved are not clear. Further study of physiological testosterone concentrations would be of considerable interest.

E. Vascular reactivity

The vascular endothelium, a single cell layer separating blood and vascular smooth muscle, regulates vessel tone through release of vasoactive factors such as NO, endothelins, and prostanoids (214). As a cellular plane, it forms the surface on which blood cells, vessel wall growth, and adhesive factors interact to form the nidus of atherogenic lesion. The pivotal significance of endothelial NO release causing vasodilatation has facilitated the development of endothelial function tests. These tests are based on comparing the vascular effects of stimuli that cause endothelial NO release (endothelium-dependent stimuli) with endothelium-inde-
due to significant differences in vascular reactivity between men and women, although the decline starts earlier in men (220). The menstrual cycle (218). FMD declines with age in both men and women (218, 219) except for higher values during the estrogen-dominated follicular and luteal phases of the menstrual cycle (218).

Both endogenous and exogenous testosterone impair vasodilatory effects similar to those reported in non-androgen-deficient men with coronary artery disease, acute coronary heart disease (217), but it is also influenced by other variables that must be controlled for studies of FMD (214). The development of a noninvasive test for arterial endothelial function (216) has allowed wider investigation of endothelial dependency stimuli that deliver NO directly to vascular smooth muscle, bypassing the endothelium. The difference in effect of endothelium-mediated NO release and after reactive hyperemia (where shear stress causes endothelial NO release) and administration of sublingual nitroglycerin represents the effect of endothelial-independent stimulus (215). The difference in effect of endothelium-independent stimulus (215) but is still controlled by other variables that must be controlled for studies of FMD (214). 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the groups at baseline, despite randomization. On the contrary, supraphysiological androgen dosage in bodybuilders is associated with reduced vascular reactivity (226, 227). Because this was equally true for bodybuilders who did not use androgens (compared with sedentary controls; Ref. 226), it may be that intense exercise rather than androgens caused reduced vascular reactivity in bodybuilders (48).

In healthy, community-dwelling older men (over the age of 60 yr) with mild age-related lowering of blood testosterone concentrations, administration of transdermal testosterone for 1 yr (228) or recombinant human chorionic gonadotropin (229) or transdermal DHT gel (230) for 3 months had no significant effect on vascular reactivity. The latter study using DHT, a nonaromatizable androgen, indicates that the unimproved vascular reactivity in the two studies using testosterone was unlikely to be due to the balancing effect of estradiol via aromatization from testosterone. These findings in older men are best explained by their not being sufficiently androgen deficient, or the treatments not being used to produce sufficiently high androgen exposure, to change vascular reactivity. Alternatively, the effects of age may not be reversible by modest net changes in androgen exposure.

By contrast, vascular reactivity is increased by long-term administration of high-dose estrogens to genetic males (M2F transsexuals; Refs. 231–233), by 8-wk oral estradiol valerate treatment in older men castrated for advanced prostate cancer (234), and by parenteral administration of low-dose estradiol to healthy young men (235). Acute administration of estradiol had no effect in healthy young men (236), and FMD was absent (although nitrate- and estradiol-induced dilatation was present) in a young man with a nonfunctional, mutated ERα gene (237). These findings that estradiol, mediated probably via ERα in endothelial cells, augments vascular reactivity in men of all ages suggest that aromatization contributes to the vascular reactivity effects of exogenous and endogenous testosterone.

Administration of testosterone to women has less clear results. Genetic females having long-term administration of testosterone (F2M transsexuals) maintaining male blood testosterone concentrations have larger brachial artery diameter and reduced nitrate-induced response, but FMD was not significantly different from age-matched female controls (238). For estrogen-treated postmenopausal women having parenteral testosterone (producing a 5-fold elevation to supraphysiological blood testosterone concentrations for women), FMD was apparently increased, although the small effect size was difficult to interpret due to baseline mismatch for vascular reactivity (239). The effects of age, aromatization, and testosterone dose in these contrasting findings remains unclear.

V. Coronary Artery Disease

The relationship of androgens to coronary artery disease has been well reviewed (240–242). Observational studies show a consistent inverse relationship between endogenous testosterone and adverse cardiovascular events (240, 243–246). These findings may be interpreted as suggesting the testable hypothesis that chronically lowered blood testosterone may increase risk of cardiovascular disease. However, such findings from cross-sectional studies cannot distinguish the direction of causality (either of which is plausible) or exclude a common cause. The opposite interpretation is that blood testosterone is mildly lowered due to heart disease, as occurs for many chronic disorders (247–250). Longitudinal studies do not support the predictive value of lowered blood testosterone concentration for further cardiovascular events (240, 251, 252), which favors the decrease in cross-sectional studies being an effect, rather than a cause. Nevertheless, the only definitive test of this important concept is a prospective interventional study of sufficient power and duration of surveillance to estimate or exclude an important protective effect size.

An important predictive relationship for blood DHEA sulfate (DHEAS) with further cardiovascular disease was first reported by the Rancho Bernardo study, which found that low blood DHEAS concentration predicted cardiovascular disease 12 yr later among community-dwelling older men, but not women (253). Subsequent observational studies confirmed these findings, albeit with a lower risk (252), whereas other similar prospective studies with more than 5-yr follow-up fail to confirm the original observation (254–256). Further analysis of the original study cohort at 19 yr has attenuated and qualified the risk (257). The apparent protective effects of DHEA remain hard to explain. DHEA and its sulfated ester DHEAS are weak androgens of adrenal origin that circulate at high blood levels in young adults before undergoing a steep decline from the third decade of life. Yet, DHEA has no convincing hormonal effects in its own right (other than due to conversion to bioactive steroids), consistent with the absence of a specific DHEA receptor or distinctive functional effects. Preliminary reports of DHEA binding (77, 258–260) have not yet led to characterization of a specific DHEA receptor. However, given the plethora of orphan receptors in the nuclear receptor gene superfamly (261), a specific DHEA receptor cannot be discounted. Similarly, there is evidence for DHEA interacting with neurotransmitter (N-methyl-D-aspartate α, γ-aminobutyric acid) receptors (262), having vasodilator properties (192, 205, 263) and many other nonspecific biological effects (264), but none that convincingly explains cardioprotective effects. Although mechanistic studies have still not identified plausible explanatory mechanisms for protective effects of DHEAS, further studies are warranted. Alternatively, methodological factors may be the explanation. For example, the very steep age-related decline in DHEAS concentrations and complex interrelationships with other age-related cardiovascular risk factors may be difficult to fully account for confounding by age despite sophisticated analyses (252). It remains to be clarified whether predictive effects of DHEAS on cardiac mortality reflect confounding by the strong age dependence of its circulating concentration (265) or other factors such as cardiac failure, which lowers DHEAS in proportion to its severity (266).

Interventional studies of androgen effects on symptomatic coronary artery disease are limited (Table 2). Three RCTs of chronic androgen therapy involving objective clinical cardiovascular end-points have been reported. Earlier studies having no controls (267) or only ad hoc controls (268, 269)
reported that androgen therapy improved symptomatic angina (270). The three well-controlled studies consistently report improvement in objective measures of cardiac ischemia (exercise stress testing) with subjective improvements with 2- to 12-wk testosterone therapy. The first study randomized 50 symptomatic men with cardiographic evidence of ischemia (postexercise ST segment depression) to weekly injections of testosterone cypionate (200 mg) or oil vehicle for 8 wk (271). Testosterone caused a highly significant reduction (32% at 4 wk, 51% at 8 wk) in postexercise ST segment depression compared with placebo, which had no effect (<2%). These findings were confirmed by a placebo-controlled, crossover study of 62 older men with established ischemic heart disease randomized to treatment with either testosterone undecanoate (120 mg/d for 2 wk, then 40 mg/d for 2 wk) or placebo, and then crossed over to the other treatment after a 2-wk washout period (272). Dramatic improvement in cardiac ischemia in subjective (77% vs. 7% with angina symptoms) and objective criteria (electrocardiogram, 69% vs. 8%; Holter, 75% vs. 8%), but no change in cardiac function (echocardiography) was observed. This latter study was apparently unblinded, and the objective scales were not described; in addition, another report gives an inconsistent account of the placebo treatment (273). Most recently, a third study involved 46 men with stable angina randomized to receive daily testosterone (5 mg) transdermal patch or matching placebo, which continued throughout the study, although an effect of the lower testosterone concentrations (224). These findings are consistent with neurogenic factors (149, 186, 192, 197).

Testosterone significantly increased time to ST segment depression during exercise stress testing, but there was no change in time to angina, magnitude of ST depression, total exercise time, or hemodynamic parameters. The second study (276) used a similar design (2.5 mg testosterone or ethanol vehicle placebo infused iv for 5 min, 2-d washout before crossover) with six men having baseline blood testosterone concentrations below the young eugonadal range. Testosterone significantly increased time to ST segment depression and total exercise time during exercise stress testing performed 30 min after infusion. Blood testosterone concentrations were elevated 54-fold, and heart rate was increased. In the third study, 32 men with treated exercise-induced myocardial ischemia (277) were randomized to receive iv testosterone (1 mg/ml) or placebo infusions for 20 min before crossover to the other treatment 7 d later. Testosterone dose was titrated to maintain serum testosterone concentrations at two times and then six times the baseline concentration. Testosterone had no effect on symptomatic angina, hemodynamic parameters, electrocardiogram, or visual or quantitative evaluation of sestamibi SPECT myocardial perfusion images compared with placebo during exercise or adenosine stress testing to provoke myocardial ischemia. The lack of testosterone effect in this study is most likely due to the continued use of antiischemic drugs during the study, although an effect of the lower testosterone concentrations achieved (6-fold elevation) cannot be excluded. The objective benefits in the two studies with highest testosterone doses suggest an organic effect, although the mood-elevating effects of testosterone may have contributed.

Acute effects of testosterone on coronary vasodilatation have been studied in three careful crossover studies of ultrashort duration (5–20 min) in men with stable angina (275–277). Two showed objective improvement in cardiac ischemia (exercise or drug stress testing) with industrial doses of testosterone. The first two studies (275, 276) each recruited 14 men and discontinued antianginal therapy prior to evaluation, whereas the third study (277) was parallel in design and antianginal therapy was continued throughout the study period. In the first study, men with a serum testosterone less than 11 nm were randomized to receive 10-min infusions of either iv testosterone (2.3 mg) or ethanol vehicle (60%) with a crossover to the other treatment 7 d later (275). With mean testosterone concentrations 22-fold higher than normal, testosterone significantly prolonged time to ST segment depression during exercise stress testing, but there was no change in time to angina, magnitude of ST depression, total exercise time, or hemodynamic parameters. The second study (276) used a similar design (2.5 mg testosterone or ethanol vehicle placebo infused iv for 5 min, 2-d washout before crossover) with six men having baseline blood testosterone concentrations below the young eugonadal range. Testosterone significantly increased time to ST segment depression and total exercise time during exercise stress testing performed 30 min after infusion. Blood testosterone concentrations were elevated 54-fold, and heart rate was increased. In the third study, 32 men with treated exercise-induced myocardial ischemia (277) were randomized to receive iv testosterone (1 mg/ml) or placebo infusions for 20 min before crossover to the other treatment 7 d later. Testosterone dose was titrated to maintain serum testosterone concentrations at two times and then six times the baseline concentration. Testosterone had no effect on symptomatic angina, hemodynamic parameters, electrocardiogram, or visual or quantitative evaluation of sestamibi SPECT myocardial perfusion images compared with placebo during exercise or adenosine stress testing to provoke myocardial ischemia. The lack of testosterone effect in this study is most likely due to the continued use of antiischemic drugs during the study, although an effect of the lower testosterone concentrations achieved (6-fold elevation) cannot be excluded. The objective benefits in the two studies with highest testosterone doses suggest an organic effect, although the mood-elevating effects of testosterone may have contributed.

Direct arterial infusion of testosterone causes acute coronary vasodilatation. Intracoronary infusions of testosterone (3 min) at physiological concentrations induces coronary artery dilatation and increased coronary blood flow in men with established coronary artery disease (278). Intravenous testosterone infusions can acutely enhance brachial artery flow-mediated dilatation, but only at supraphysiological concentrations (224). These findings are consistent with numerous experimental findings that testosterone causes acute coronary dilatation (149, 186, 192, 197).

In summary, these findings suggest that, at high doses,
testosterone is a coronary vasodilator with effects comparable to conventional anti-ischemic drugs. None of the clinical studies clarify the involvement of aromatization, 5α-reduction, AR, or nongenomic mechanisms in testosterone effects. Unless the vasodilator properties of testosterone extend to lower concentrations (206), these findings cannot explain the clinical trial findings with lower testosterone doses, and additional effects may be involved. Whether these findings with high-dose testosterone have practical implications for development of a novel anti-ischemic therapy is uncertain. On the basis of existing epidemiological findings, the hypothesis that testosterone may ameliorate progression of cardiac ischemia warrants further clinical studies.

VI. Hypertension

The role of reproductive hormones and gender differences in hypertension has been well reviewed recently (279, 280). Blood pressure is higher in men than women from puberty onward (281, 282) so that the prevalence and complications of hypertension exhibit consistent gender differences. Animal models suggest that this is primarily due to testosterone increasing blood pressure, but the mechanism remains only partly understood. In men, small studies of older men castrated for prostate cancer show increased large artery stiffness (283, 284) but no consistent changes in blood pressure (221, 283–285). It is possible, however, that aging effects overshadow those of testosterone. Epidemiological findings that testosterone and blood pressure are inversely related in populations of men (251, 286) are supported by metaanalysis (287) and other study (249) findings that chronic diseases, including hypertension, accelerate the age-related decline in blood testosterone concentration. With advancing age, blood pressure rises progressively so that by the seventh decade blood pressure and prevalence of hypertension are similar in men and women. The absence of blood pressure changes in longitudinal studies of the menopausal transition, together with the minimal effects of estrogens on blood pressure (280), suggests that catch-up in older women’s blood pressure may reflect an effect of age rather than estrogen deficiency.

In untreated hypertensive men, sexual function (288) and blood testosterone concentrations (288–291) are mildly reduced within the eugonadal reference range. There is a high prevalence of erectile dysfunction (ED) among treated hypertensive men, an adverse effect that reduces quality of life and therapeutic compliance (292). Although all classes of antihypertensive drugs have been linked with ED, the most frequent mechanism appears to involve unrecognized atherosclerotic pelvic arterial insufficiency, which reduces cavernosal blood inflow particularly after lowering of systemic blood pressure regardless of antihypertensive drug class (293). Prospective studies of hypertensive patients starting antihypertensive therapy confirm that most drugs can reduce sexual activity (294, 295) and blood testosterone concentrations. These small reductions in blood testosterone concentration are most probably the consequence, rather than the cause, of reduced sexual activity (296). Whether any antihypertensive drug class has consistent benefit in ED risk, at equivalent hypotensive efficacy, remains to be established in prospective controlled studies with careful documentation of sexual function before and after treatment.

All major rat models for hypertension (SHR, Dahl salt-sensitive, deoxycorticosterone acetate-induced, NZ genetically hypertensive) show consistently that males have higher blood pressure than age-matched females. Hypertension is prevented by orchidectomy (but not oophorectomy) of young hypertension-prone SHR rats and is reproduced by testosterone treatment of castrate males or females (279, 280). Introducing the tfm X chromosome (including a mutated, nonfunctional AR) into the SHR model abolishes the gender difference in blood pressure (11), as does flutamide but not finasteride (297), indicating involvement of the AR but not 5α-reduction. Neonatal androgen imprinting determines the sexually dimorphic pattern of blood pressure as a single neonatal testosterone dose administered to newborn females increased their blood pressure to male levels at maturity (298). The nature of the testosterone effect remains elusive, although kidney transplantation demonstrates the involvement of an extrarenal factor (12), an endothelium-derived substance causing hyperpolarization of vascular smooth muscle involving potassium channels (13). Evidence for key involvement of the renin-angiotensin system, based on effects of an angiotensin I-converting enzyme (ACE) inhibitor (299), is less convincing given the nonspecific hypotensive effects of ACE blockade, and further studies using transgenic models would be more definitive. The hypothesis that the role of testosterone in hypertension-prone rats may reflect variations in tissue androgen sensitivity (279) warrants further analysis, including the association of the CAG triplet repeat functional polymorphism in the human AR that influences tissue androgen sensitivity (300). Similar findings are reported for the salt-sensitive hypertensive male rat model with testosterone regulating renal α2a adrenoceptors (301). There is also evidence for a rat Y chromosome locus producing gender-selective hypertension mediated by testosterone and the AR (14) and involving sympathetic nervous system activation (302). The responsible rat locus, its human homolog, and the specific mechanisms remain to be identified.

Few studies have examined the gender differences in mouse blood pressure. Gonadectomy reduces blood pressure in mature male but not female mice (303) and abolishes the gender difference in plasma ACE activity (304) and cardiac ventricular ACE mRNA (305). Further studies using the wealth of genetic tools available to study molecular physiology of blood pressure in the mouse are becoming available (306). Well-developed technologies exist to introduce transgenes with control of spatial and temporal patterns to create global, tissue- or cell-specific gene inactivation with or without triggering of gene inactivation by molecular switches (306). Additionally, genotypes can be modified or rescued by crossbreeding with other informative genotypes providing unique physiological precision and incisiveness. Further application of mouse genetics to understanding the sexually dimorphic renin-angiotensin system and its role in the maintenance of normal and elevated blood pressure is likely to be highly informative on how gender and androgens modulate this system (307).
VII. Cardiac Hypertrophy and Failure

The effects of gender and reproductive hormones on cardiac mass have been reviewed (308). Even after allometric adjustment for gender differences in overall body size (itself androgen dependent), left ventricular mass begins to differ between men and women from puberty onward and continues to diverge throughout life (308). The latter remains controversial because of the complexity of accounting for age-dependent accumulation of pathologies (both clinically overt and subclinical) that influence cardiac mass (e.g., ischemia, hypertension). This has led to the hypothesis that testosterone influences human left ventricular hypertrophy (309).

A variety of rat models for cardiac hypertrophy and heart failure involving pressure (aortic banding) or volume (fistula) overload report males showing poorer survival and cardiac function adaptation (310). After experimental myocardial infarction, testosterone is reported to cause both cardiac hypertrophy and improved cardiac function (311) or high risk of acute cardiac rupture and worse cardiac function (312). Further studies standardizing the cardiac lesion and androgen regimen as well as evaluating the role of testosterone metabolites and AR are required to clarify and extend these findings. A seminal study of the latter issues highlights the emerging importance of local testosterone metabolism in modulating tissue-specific androgen action (313). This systematic review of cytochrome P-450 monoxygenase gene expression and activity in human and rodent (SHR) cardiac hypertrophy observed increases in aromatase (CYP19) and 5α-reductase (but not other steroid metabolizing enzymes 3β- and 17β-hydroxysteroid dehydrogenase isoforms) as well as AR. Hence, net androgen action in the heart and with cardiac disease may be influenced by tissue-specific variations in AR, androgen activating enzymes as well as AR coregulators, providing a range of novel therapeutic targets.

Genetic mouse models for cardiac hypertrophy and failure are being developed (314), but at present little is known regarding the role of gender or androgens in the pathogenesis of cardiac hypertrophy or failure. A gender difference in ventricular repolarization rate due to a potassium channel (Kv1.5) is reported to be androgen dependent in mice (315).

Blood testosterone concentrations are reduced in men with cardiac failure of any etiology, depending on the severity of the chronic heart failure. In end-stage cardiac failure, lowered blood testosterone concentration is improved with chronic mechanical circulatory support (316), whereas in men with stable cardiac failure, the reduction in blood testosterone concentrations is modest (317) or nil (318, 319). Although these changes may represent the hypothalamic reaction to severe chronic illness (320), a preliminary report of a pilot study that randomized 20 men with chronic heart failure to receive weekly injections of testosterone enanthate (100 mg) or placebo for 12 wk found that testosterone treatment significantly improved left ventricular ejection fraction and exercise capacity compared with placebo (321). A larger placebo-controlled study of transdermal testosterone study is under way (T. H. Jones, personal communication), and the effects of androgens on the cachexia of severe cardiac failure (322) would be of interest.

VIII. Cerebrovascular Disease

A key issue in understanding the relationship of androgens to extracardiac atherosclerosis is the assumption of a unitary atherosclerotic disease process in all arteries. This is supported by the similar pathological appearances of all stages at different sites (323–325), the common localization of plaques in areas of arterial wall stress and stretch (326), and similar risk factor patterns for atherosclerosis in different arterial trees (327). Doubts about this assumption arise chiefly from differences in relative importance of specific risk factors from individual observational studies (328), but systematic differences appear to be few.

The age-adjusted prevalence of stroke is 1.25- to 2-fold higher in men than in women (329–331). Like coronary artery disease, cerebrovascular disease increases with age, with a doubling of stroke rates in both man and women for each decade after the age of 55 yr (331). Although data are sparse, there is no evidence of acceleration in stroke incidence specifically related to menopause rather than to advancing age. Whereas coronary artery disease has largely singular (thrombosis) pathology, cerebrovascular disease (stroke) can be due to either thromboembolism (~75%), the cerebral manifestation of generalized atherosclerosis, or hemorrhage (~25%), which is more related to genetics and hypertension. Thus, the role of androgens in stroke pathogenesis may differ according to the underlying pathology. Nevertheless, the minority of population-based studies that examine specifically ischemic stroke (determined at autopsy or by radiological imaging) confirm that men have more ischemic strokes than women (332, 333).

In case-control studies, male stroke survivors have lower blood (334–336) and cerebrospinal fluid (335) testosterone concentrations, similar to observations after myocardial infarction (240). Whether lowered blood testosterone reflects a preexisting state predisposing to stroke or an acute reaction to stroke is not known. This issue could be clarified by the predictive value of blood testosterone concentration for stroke from ongoing prospective studies. Although prospective data are not yet available, a cross-sectional population-based study found that endogenous testosterone was inversely related to carotid intima media thickness (337), a strong predictor of subsequent stroke and other atherosclerotic vascular events (338). Stroke is associated with high doses or abuse of synthetic androgens with case reports in three athletes and four men with hypoplastic anemia by 1993 (339) and another five, including two with underlying medical disorders subsequently (340–344). Only a single case associated with testosterone is reported, involving overdose in a hypogonadal adolescent (345). The unknown denominator of usage from which these cases are drawn makes it difficult to judge the actual clinical risks of stroke associated with androgen use, particularly with standard testosterone doses.

Only one well-controlled clinical study has examined the effects of testosterone on cerebrovascular disease. Forty postmenopausal women receiving stabilized estradiol/progesterin therapy were randomized to receive either oral testosterone undecanoate (40 mg/d) or no treatment in a nonmasked study for 8 months (346). Five of the 20 (25%) women on
testosterone withdrew due to androgenic side effects or independent medical advice. Testosterone treatment, producing a 4-fold increase in blood testosterone, produced a 6% increase in the pulsatility index of the middle cerebral artery, but not of the internal carotid artery. The clinical significance of this small increase in middle cerebral artery resistance is unclear. No controlled studies of androgens with cerebrovascular end-points in men are reported.

The effects of testosterone on cerebral vasculature are poorly defined. One rat study reported that testosterone increases the autoregulatory myogenic tone of cerebral arteries through an endothelium-dependent mechanism independent of NO synthase and possibly mediated by other vasoactive substances (e.g., prostanooids or endothelins) that inhibit vascular smooth muscle K channels (347). Effects of higher (micromolar) concentrations of testosterone, which are vasodilatory in all other arterial beds studied, have not been reported in the cerebral vasculature.

Studies of stroke-prone SHR rats report that males had higher blood pressure and stroke rate and shorter life span than females (348). These outcomes were not influenced by castration; estrogen treatment of castrate males reduced stroke rate and lengthened life span to match female levels, whereas testosterone treatment of females reproduced intact male stroke rate and life span (348). These findings are consistent with evidence that hypertension in SHR rats is testosterone mediated (see Section VI), and stroke-proneness is due largely to hypertension.

Estrogen-mediated neuroprotection has been described (349, 350), including in male rats where it is independent of endogenous testosterone (351). Testosterone enhances neuronal survival and regeneration after ischemic and toxin-mediated damage (352–356), effects that involve aromatization to estradiol (357) exploiting the neuroprotective effects of the phenolic A ring (358). On the contrary, however, it is claimed that ischemia-reperfusion injury (359, 360), in which reactive hyperemia after reperfusion leads to vascular damage and leakage, might involve vasodilatory effects of testosterone (360). This seems unlikely given the high testosterone concentrations required for vasodilatation together with the profound reduction in blood testosterone concentrations during acute illness (361). Nevertheless, because acute androgen blockade appears to improve recovery from trauma and shock (362), studies of pharmacological androgen blockade might be of interest.

In summary, whether the lowered blood testosterone concentrations in male stroke survivors reflect a cause or effect of stroke remains unclear. This should be resolved by prospective studies of the predictive value of blood testosterone concentrations for future stroke. A significant predictive value might justify a placebo-controlled trial of androgen therapy of modest duration in a high-risk population aiming to reduce stroke risk.

IX. Peripheral Arterial Disease

Peripheral arterial disease (PAD), as the manifestation of atherosclerotic arterial disease affecting the pelvis and legs, has been comprehensively reviewed recently (363, 364). Although it is generally assumed that the atherosclerotic pathogenesis of PAD is similar to that in the cardiac and cerebral circulations, fewer observational or interventional studies of PAD are available to evaluate this empirically. This reflects the neglected clinical status of PAD, which remains undertreated (365, 366) despite high mortality and morbidity (363, 364), a perspective also reflected in the limited contributions to research on atherosclerosis in this area.

Observational studies show a crude prevalence of PAD that is 2- to 3-fold higher in men than women (363) and even higher (6-fold) for abdominal aortic aneurysm (367) in larger, population-based studies. The higher gender disparity in abdominal aortic aneurysm may be due to more sensitive and specific detection using ultrasound in larger population studies. When analysis of PAD is restricted to studies using standardized diagnostic criteria for asymptomatic (ankle-brachial index) or symptomatic (World Health Organization/Rose questionnaire for intermittent claudication) disease in population-based studies (rather than high-risk subpopulations), the age-specific prevalence is closer in men and women, but it ranges from 3–19% between centers (368). The weaker evidence for a gender disparity gap in PAD is more likely to reflect fewer high-quality studies rather than any fundamental differences in pathogenesis of PAD compared with cardiac and cerebral vascular atherosclerosis.

Interventional studies on the role of androgen therapy in PAD are limited and largely negative. Empirical androgen therapy to treat claudication was first used over six decades ago (267, 369), soon after the first clinical availability of testosterone (370). Four randomized controlled studies performed over 30 yr ago are consistent in showing no benefit for androgen therapy in PAD. The first study randomized (by alternate allocation) 39 men with claudication to treatment with testosterone enanthate (200 mg/wk for 3 wk, then fortnightly) or placebo oil vehicle injections for 6 months (371). Treatment produced no significant benefit over placebo in symptoms, walking time to onset of symptoms, handgrip strength, venous filling time, pulse amplitude or side effects, despite significant improvement in leg muscle blood flow (Xenon clearance) and increased libido. The second double-blind study randomized 44 nondiabetic men with claudication to receive a lower androgen dose (testosterone isobutyrate, 300 mg) or control (meprobamate as a placebo) injected every 14 d over a 12-wk study period before crossover to the other treatment (372). Testosterone treatment did not alter foot pulses, metronome walking distance (a measure of claudication), plethysmographic parameters, or symptoms, although body weight was increased. However, carryover effects leading to a null bias may not have been excluded by a washout period, the length of which was not stated. A third study randomized 26 patients to injections of 100 mg testosterone propionate or placebo oil vehicle three times weekly for 2–6 months without any subjective or objective benefit (373). Finally, the fourth double-blind study treated 22 nondiabetic men with claudication with either testosterone (100 mg/d plus various other hormones 6 d/wk) or placebo vehicle injections for 6 wk (374). No randomization was specified, and no difference in pain or standardized walking test was reported. In summary, the few available controlled studies from three decades ago lack so-
phisticated study design or methodology by current standards (375), but nevertheless androgen therapy has demonstrated neither significant benefit nor much promise in treatment of PAD.

Comparing the placebo-controlled clinical trials, there is a striking discrepancy between these consistently negative findings for testosterone treatment and the equally consistent alleviation of cardiac ischemia by testosterone. This may reflect different caliber vessels involved with coronaries possessing more dynamic vasodilatory capacity compared with the larger aortoiliac and femoral vessels in PAD. If so, this also favors the interpretation that beneficial testosterone effects in myocardial ischemia are more likely due to reversal of cardiac ischemia, rather than nonspecific mood elevation or improved pain tolerance because the latter might be equally likely in claudication.

**X. Erectile Dysfunction**

Vasculogenic ED, the most prevalent cause of erectile failure, has a strong but insufficiently appreciated relationship to atherosclerosis. The penis is a highly specialized vascular bed, and erection is a vascular hydraulic mechanism activated by neural triggering that coordinates arteriolar dilation, smooth muscle relaxation, and venous constriction (376). This mechanism involves NO release (377), increased cytosolic cyclic GMP (378), and decreased calcium (379) in smooth muscle cells leading to vasodilatation and cavernosal filling (380). Medical treatment of ED was revolutionized by the advent of a highly effective oral drug (sildenafil; Refs. 380 and 381) targeting this pathway through the inhibition of tissue-selective, type 5 phosphodiesterase inhibitor that enhanced cyclic GMP action via inhibition of its metabolism (382).

ED is highly age-dependent and remarkably frequent from midlife onward. Among American men aged 40–70 yr, approximately 10% have severe ED, and approximately 50% have some degree of ED (383). ED is now understood to be mostly due to organic, primarily vasculogenic, rather than psychogenic factors or androgen deficiency. A major factor in vasculogenic ED is arterial insufficiency due to the pelvic arterial variant of atherosclerotic vascular disease involving aortoiliac (Leriche syndrome), smaller conductance vessels together with endothelial dysfunction (384). Animal models show conclusively that atherosclerosis of the lower aorta, iliac, and penile arteries causes erectile failure in rabbits (385) and monkeys (386). The close relationship of ED to PAD is reflected by the efficacy of a type 3 phosphodiesterase inhibitor, cilostazol, in medical therapy of claudication (387, 388).

Major risk factors for ED, notably aging, smoking, hyperlipidemia, hypertension, and diabetes, are similar to those for cardiovascular disease (384, 389). Consequently, men with ED have higher risk for other atherosclerotic diseases, and men with coronary, cerebral, or peripheral atherosclerotic vascular disease are at higher risk for ED (390). This suggests the following: 1) ED may be a sentinel feature of atherosclerosis with prognostic significance for men’s general health (391), and 2) primary prevention may retard progression of ED (392). Whether lipid-lowering pharmacotherapy can prevent ED is clouded by claims that the most effective agents (statins) cause ED as a common side effect (384), although available evidence is inconclusive (393–395) because evaluating causality for drug-associated ED is frequently problematic without thorough characterization of pretreatment erectile function. This limits the utility of secondary analyses of completed large-scale placebo-controlled statin studies unless new-onset ED can be referred to an appropriate denominator with well-characterized erectile function. Hence, formal evaluation of whether ED is a sentinel event for generalized atherosclerosis requires a large-scale, randomized, placebo-controlled prospective study.

The relationship of androgens to vasculogenic ED might be considered similar to that with atherosclerosis elsewhere, but this is largely overshadowed by the stronger relationships of androgens to aspects of sexual function other than erection. The primary role of androgens in human male sexual function is in maintenance of libido (396, 397). Androgen effects on human erectile function are more complex, with spontaneous nocturnal erections being more androgen-dependent than psychogenic erections (398). In experimental animal models, there is evidence that androgens influence pelvic autonomic nerves (399) and penile vasculature and smooth muscle (400). In humans, however, there still remains scant evidence regarding direct androgen effects on the neurovascular mechanisms subserving erectile function (400), a limitation that partly reflects the difficulty of access to relevant human tissues. Excluding the small minority (<5%) of men presenting with ED due to androgen deficiency from pituitary or testicular pathologies (401), most men with ED have normal or minimal reduction in blood testosterone concentration. The small reduction in blood testosterone concentration among men with ED who are not frankly androgen deficient may reflect sexual inactivity itself rather than its cause (296). As a result, empirical testosterone treatment in non-androgen-deficient men with ED may activate sexual behavior without improving erectile function or satisfaction compared with placebo (402). Particularly outside clinical trial settings, an unrecognized but strong placebo response is likely to be invoked. Such widening of the gap between libido and performance may even be considered mental cruelty, rather than compassionate medical care.

The role of endogenous or exogenous testosterone in the rate of progression of vasculogenic ED remains difficult to study. Endothelial dysfunction, involving disrupted release of vasoactive mediators from the vascular endothelium, is an important early feature of ED as well as an important predictor and pathogenic mechanism in cardiovascular disease. Both forms of endothelial dysfunction have been linked by the hypothesis that defective NO activity may be a common mechanism (403). A preliminary evaluation in men with diabetes found a relationship between biochemical indices of endothelial function (thrombomodulin, cell-adhesion molecules) with ED (404). Further evaluation of this hypothesis using more specific and sensitive hemodynamic measures of peripheral endothelial dysfunction (FMD) in men with ED may shed light on any role of androgens in the progression of ED. Whether androgen-mediated inhibition of FMD in brachial vessels has any counterpart in the endothelium of
human penile vasculature and cavernosal smooth muscle remains to be studied further (405).

In summary, penile erection is a neurovascular hydraulic mechanism operating in a highly specialized arterial system. Atherosclerosis involving this arterial bed is the most frequent cause of ED, among the most common and distressing pathologies afflicting men’s later life. Considering its highly motivating potential, an issue with important public health ramifications for prevention of cardiovascular disease is whether ED can be considered a sentinel event predicting wider atherosclerotic complications and whether pharmacological antiatherogenic therapy may prevent or reverse vasculogenic ED. Overt androgen deficiency is rarely the cause of new presentations of ED and androgen replacement therapy has little role in the treatment of ED. The role, if any, of androgens in progression of vasculogenic ED is little understood and difficult to study because it is largely overshadowed by the primary effect of androgens on sexual function being on other aspects of sexual function.

XI. Conclusion: the Present and Future

In recent years, there has been a dramatic increase in research into androgen effects on the cardiovascular system. Yet, present knowledge of the role of androgens in cardiovascular physiology and disease remains fragmentary, and the best available evidence is mostly far from complete. Whereas previously androgens were considered harmful (and estrogens protective) for the cardiovascular system, current empirical findings give a far richer but more complex, and at times confusing, picture. Considerable evidence suggests that androgens have beneficial or neutral cardiovascular effects and that they exert different effects at early (plaque formation) and late (rupture, thrombosis, vasospasm) stages of atherosclerosis. Patterns of age-specific cardiovascular death rates provide little support for the gender gap being due to estrogen protection. Rather, hormonal effects may provide a head start in men at an early stage, but the subsequent tempo of atherosclerotic progression is similar in men and women. Such early hormonal effects may involve perinatal androgen imprinting or an early stage of atherosclerosis.

The estrogen protection hypothesis has been refuted, at least for oral estrogens, in men as it has in women more recently. Whether nonoral administration of tissue-specific partial estrogen agonists (selective estrogen receptor modulators), avoiding the oral route with its unavoidable first-pass hepatic overdosage, would produce more favorable outcomes is open to speculation. Considering all available evidence on androgen effects, current findings on sex hormones and heart disease better fit the hypothesis of Barrett-Connor (244) that isosexual hormone deficiency may be harmful for the cardiovascular system, whereas gender-appropriate, isosexual hormone replacement by physiological means may be beneficial or may at least reverse deleterious effects of hormone deficiency. The adverse effects of androgen abuse on the cardiovascular system remain largely anecdotal, and systematic case-control studies of former power athletes and other abusers are needed.

In vascular cells, testosterone action often involves nongenomic effects on vascular smooth muscle and sometimes aromatization for endothelial-mediated effects. Knowledge of local fine-tuning of vascular cell androgen sensitivity, in different arterial beds and under different physiological and pathological conditions, by genomic and hormonal mechanisms still remain to be defined. Advancing knowledge in this area seems likely to provide not only valuable physiological insight but also novel therapeutic approaches based on tissue-specific nonsteroidal androgens. Nongenomic vasodilator effects of high testosterone concentrations partly explain objective improvement in exercise-induced cardiac ischemia. By contrast, testosterone treatment does not improve PAD or ED, whereas androgen effects on cerebral vessels have been little reported. Testosterone is a major pathogenic factor in experimental rodent models of hypertension, but the corresponding evidence in humans is too limited to identify any specific role of androgens in human hypertension or heart failure.

In the future, the promising high-impact areas for further research include a characterization of the nongenomic mechanisms of androgen effects, identification of androgen target genes, the wider use of gene targeting in mouse models using temporal and spatial control of gene expression to dissect complex pathways of vascular cell interactions, the regulation and dynamic roles of AR coregulators and other determinants that modulate tissue androgen sensitivity, and the development of tissue-specific nonsteroidal androgens. Among key clinical areas, the role of adjuvant androgen therapy, antiandrogens, and especially tissue-specific partial androgen agonists (selective AR modulators, designer androgens) for stable ischemic heart disease, heart failure, hypertension, and slowing progression of native and graft atherosclerosis is promising areas. With regard to clinical trials of androgen therapy in aging or other clinical settings, variations of testosterone dose or blood concentrations within the physiological male range have no predictable deleterious short-term or medium-term cardiovascular effects. This indicates sufficient safety for androgen use in controlled clinical trials in aging and other emerging applications of androgen therapy. Yet, the long-term safety of androgens is not sufficiently established for wider usage in male aging to be considered an acceptable risk for population health. Still less well defined is the safety of androgen abuse, particularly long-term.

The recent flourishing of research into androgen effects on the cardiovascular system has fostered new concepts and approaches that promise to provide greater insight into the basis of the gender gap in cardiovascular disease and androgen action in general, as well as greater confidence in how to ensure safety in the widening opportunities for adjuvant use of androgens in medicine.

Acknowledgments

We acknowledge the intellectual stimulation and long-term collaboration of our cardiology colleague Prof. David Celermajer. We thank an anonymous reviewer for suggesting congenital adrenal hyperplasia as a natural model for perinatal androgen imprinting in females.

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Endocrine Reviews, June 2003, 24(3):313–340

The endocrine control of male and female reproductive functions has been the focus of much research in recent years. In particular, studies have increasingly emphasized the role of androgens in the regulation of various aspects of human health, including cardiovascular disease. This review will summarize the current understanding of the relationship between androgens and cardiovascular disease, with a focus on the potential benefits of androgen therapy in men with androgen deficiency. The review will also discuss the role of androgens in the regulation of cardiovascular function in women and the potential implications for the treatment of cardiovascular disease in postmenopausal women.

The relationship between androgens and cardiovascular disease is complex and multifaceted. Androgens play a critical role in the regulation of various aspects of cardiovascular function, including cholesterol metabolism, arterial wall function, and sexual function. In men, androgen deficiency is associated with an increased risk of cardiovascular disease, while androgen therapy has been shown to improve cardiovascular function in men with androgen deficiency.

In women, the relationship between androgens and cardiovascular disease is less clear. While some studies have suggested a protective effect of androgens in postmenopausal women, others have found no significant association. Further research is needed to clarify the role of androgens in the regulation of cardiovascular function in women.

Conclusion

In conclusion, the relationship between androgens and cardiovascular disease is complex and multifaceted. Further research is needed to clarify the role of androgens in the regulation of cardiovascular function in both men and women. The potential benefits of androgen therapy in men with androgen deficiency and the potential implications for the treatment of cardiovascular disease in postmenopausal women warrant further investigation.

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